IN VITRO MECHANICAL EVALUATION OF EQUINE LARYNGEAL TIE-FORWARD CONSTRUCTS PERFORMED WITH DIFFERENT SUTURE MATERIALS AND PLACEMENT PATTERNS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in VMS-Veterinary Clinical Medicine
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2014

Urbana, Illinois

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ABSTRACT

Surgical rostral advancement of the larynx, also known as the laryngeal tie-forward (LTF) procedure, is the treatment of choice for dorsal displacement of the soft palate (DDSP) in horses. The LTF procedure can be performed with different suture materials and different suture patterns or techniques. We investigated and compared the mechanical characteristics of in vitro LTF constructs prepared with commonly used suture materials using different techniques. Also, we compared the maximal loads at failure of the in vitro LTF constructs to the loads exerted on the sutures tightened to achieve adequate rostral advancement of the larynx in cadaver horses. There was no difference in maximal load at failure between the constructs prepared with polyethylene sutures and the constructs prepared with similarly placed polyester sutures. However, the constructs prepared with polyethylene sutures by use of the standard technique were significantly stronger than the ones prepared with polyethylene or polyester sutures combined with metallic implants. Moreover, the constructs prepared with polyethylene sutures and metallic implants rapidly approached their maximal load at failure. The suture material and technique influenced the constructs failure mode and suture elongation at failure. Despite these results, the maximal load at failure for all in vitro constructs was significantly higher than the maximal suture loads recorded in the cadaver horses. The results suggest that the LTF procedure can be performed in vivo with any of the suture materials and techniques tested in this study. However, polyethylene sutures placed with the standard technique may be clinically superior in horses with DDSP as these provided the highest mechanical strength and resistance to elongation, which may avoid retraction of the larynx and increased probability of racing after surgery.

DEDICATION

I lovingly dedicate this thesis to my wife,

my parents, and sisters, who supported

me each step of the way.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my advisor, Dr. Santiago Gutierrez-Nibeyro, for his expertise and knowledge that added considerably to my graduate experience. I also thank him for his input in many areas of my surgical training, as well as his countless hours of reflection and encouragement, and most importantly, his patience throughout the entire process.

I would like to thank my other committee members for their expertise and for being extremely generous with their time. Special thanks to Dr. Matthew Stewart for his thoughtful comments and suggestions; Dr. David Schaeffer for his promptness, expertise, and valuable assistance with the different statistical analyses performed for the study; Dr. Gerald Pijanowski for his meticulous comments; and Dr. Amy Wagoner Johnson for her input and valuable expertise with biomechanics. Also, I would like to thank Dr. Gavin Horn for his enormous intellectual and personal contributions and for always being available throughout this project. This study would not have been possible without his generous donation of his time and enthusiastic attitude operating the machinery for the mechanical testing. I owe my deepest gratitude to you. I would also like to thank Arthrex, and The Illinois Equine Industry Research and Promotion Board for their support in funding this project.

Finally, I would like to thank my family for the support they provided me through my entire life and in particular, I must acknowledge my wife and best friend Kristin, without her love, encouragement, and support, I would not have finished this thesis.

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CHAPTER 1

INTRODUCTION

Dorsal displacement of the soft palate (DDSP) is the leading cause of upper airway obstruction in racehorses, affecting 10-40% of Thoroughbred and Standardbred horses (Lane et al., 2006a; Priest et al., 2012). Recently, dysfunction of the thyrohyoideus muscle has been implicated in the pathophysiology of DDSP in racehorses (Ducharme et al., 2003). Combined rostral advancement of the larynx and replacement of the action of the thyrohyoideus muscle with prosthetic sutures, also known as the laryngeal tie-forward (LTF) procedure, can restore nasopharyngeal stability in racehorses with experimentally induced DDSP (Ducharme et al., 2003). The surgical procedure consists of anchoring the larynx in a more rostral and dorsal position to the basihyoid bone with paired nonabsorbable sutures placed between the thyroid cartilage and the basihyoid bone along the orientation of thyrohyoideus muscles (Ducharme et al., 2003; Woodie et al., 2005a). The LTF procedure has the highest success rate to restore normal airway function and nasopharyngeal stability in racehorses with intermittent or permanent DDSP (Cheetham et al., 2008; Woodie et al., 2005a).

Despite the widespread clinical use of the LTF procedure, there has been no investigation of the mechanical strength of the LTF construct. Although the incidence of suture failure is low (Cheetham et al., 2008; Ducharme, 2013; Woodie et al., 2005a), it is our experience that sutures can break in the immediate postoperative period as a result of sudden increased loading experienced during recovery from general anesthesia. Moreover, the LTF technique has been modified since the original description in an effort to increase the mechanical strength of the surgical constructs and avoid surgical failure (Ducharme, 2013; Woodie et al., 2005a). Therefore, mechanical evaluation of LTF constructs with different suture materials and different suture patterns can be informative to enhance the standard surgical technique, and assist surgeons in the selection of the most mechanically suitable suture materials and suture placement patterns to perform the LTF procedure in horses diagnosed with DDSP.

The first objective of this study was to evaluate and compare the mechanical properties (load, elongation, stiffness, and mode of failure) of in vitro LTF constructs prepared with commonly used suture materials (polyethylene or polyester sutures) using different techniques in single load to failure testing. The second objective was to compare the maximal loads at failure of the LTF constructs to the loads exerted on the sutures tightened to achieve adequate rostral advancement of the larynx in cadaver horses.

Our hypothesis were the following: 1) there would be no difference between the maximal loads at failure of the in LTF constructs and the loads exerted on the sutures tightened to achieve adequate rostral advancement of the larynx in the cadaver horses; 2) there would be no difference in maximal load at failure, elongation at failure, or stiffness between the LTF constructs prepared by used of the standard or modified technique and polyester or polyethylene sutures; 3) there would be no difference in maximal load at failure, elongation at failure, or stiffness between the LTF constructs prepared by use of the standard technique and the ones prepared by use of the modified technique with the metallic implants; and 4) there would be no difference in loads at different elongation points (15, 20, and 30 mm) among the different LTF constructs.

CHAPTER 2:

LITERATURE REVIEW

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1. Relevant anatomy and function of the upper airway

Pharynx:

The pharynx in the horse is a 15 cm long tubular structure composed of several muscle groups innervated by several cranial nerves and lined by a highly sensitive respiratory mucosa (Sisson, 1975a). The pharynx extends from the caudal portion of the nasal cavity and oral cavity to the larynx, and it is somewhat funnel-shaped rostro-caudally (Sisson, 1975a). The pharynx has numerous physiological functions including olfaction, phonation, deglutition, thermoregulation, protection, and conditioning of the inspired air. However, the most vital function of the pharynx is to serve as a conduit for air to and from the lungs because horses are obligate nasal breathers

(Derksen, 2012). Therefore, diseases that anatomically or physiologically affect the pharynx in the horse have negative consequences for the upper airway function and cause upper airway obstructions, particularly in racehorses during strenuous exercise (Holcombe and Ducharme, 2004b).

The pharynx in the horse has no osseous or cartilaginous support, however, it is supported by its muscles and a strong aponeurosis that connect the pharynx to the cricoid and thyroid laryngeal cartilages, as well as, to the palatine, pterygoid, and hyoid bones (Sisson, 1975a). Anatomically, the pharynx is divided in nasopharynx, oropharynx, and laryngopharynx. The soft palate divides the nasopharynx from the oropharynx, and the space extending from the base of the epiglottis to the cricoid cartilage (ventral to the palatopharyngeal arch) is the laryngopharynx (Ducharme, 2012). During respiration, the caudal border of the soft palate intimately contacts the subepiglottic tissues preventing communication between the oropharynx and nasopharynx (Ducharme, 2012). The pharyngeal lumen dorsal to the palatopharyngeal arch is lined with ciliated epithelium, while the pharyngeal lumen ventral to the palatopharyngeal arch is lined with stratified squamous epithelium (Sisson, 1975a). The principal anatomical relations of the pharynx include the guttural pouches, larynx, pterygoideus medialis muscle, stylohyoid bone, linguofacial trunk, glossopharyngeal nerve, rostral laryngeal nerve, hypoglossal nerve, retropharyngeal and cranial cervical lymph nodes, and external carotid artery which provides the blood supply to the pharynx (Sisson, 1975a).

The intrinsic pharyngeal muscles can be divided in three groups: the rostral pharyngeal constrictors, the middle pharyngeal constrictors, and the caudal pharyngeal constrictors (Holcombe and Ducharme, 2004b). During swallowing, the pharynx becomes a sphincter due contraction and shortening of the pharyngeal constrictor muscles to move the food bolus into the esophagus. During breathing, contraction of the pharyngeal constrictor muscles stiffen the pharynx and maintain the nasopharynx patent, particularly during the expiratory phase of the breathing cycle (Holcombe, 2013b). In addition, there are extrinsic pharyngeal muscles that affect nasopharynx function, such as the muscles of the hyoid apparatus, muscles of the soft palate and the stylopharyngeus muscle (Holcombe, 2013b).

The pterygopharyngeus muscle (rostral pharyngeal constrictor) is a flat triangular muscle that lies on the rostral portion of the lateral wall of the pharynx. It originates from the pterygoid bone dorsal to the stylopharyngeus muscle, crosses the levator veli palatini muscle, and inserts into the median pharyngeal raphe. Contraction of the pterygopharyngeus muscle shortens the soft palate and draws the larynx and esophagus toward the oropharynx during swallowing (Sisson, 1975a).

The hyopharyngeus muscle (middle pharyngeal constrictor) may consist of two different muscles in the horse: the chondropharyngeus and ceratopharyngeus muscles. The chondropharyngeus muscle is a broad and fleshy muscle that originates from the thyrohyoid bone and thyroid cartilage and inserts into the median pharyngeal raphe. The caudal part of the muscle passes under the thyropharyngeus muscle, while the rostral part of the muscle overlies the pterygopharyngeus and palatopharyngeus muscles (Sisson, 1975a). The ceratopharyngeus is a small muscle that originates from the medial distal surface of the stylohyoid bone, passes dorsally and caudally to the palatopharyngeus muscle, and inserts into the median pharyngeal raphe under the chondropharyngeus muscle (Sisson, 1975a).

Motor innervation to the pterygopharyngeus muscle and hyopharyngeus muscle is supplied from the pharyngeal plexus, which receives inputs from the pharyngeal branch of the vagus and glossopharyngeal nerves (Baptiste, 1997; Sisson, 1975a).

The thyropharyngeus muscle (caudal pharyngeal constrictor) originates from the lateral surface of the thyroid cartilage caudal to its oblique line and inserts into the median pharyngeal raphe (Sisson, 1975a). The cricopharyngeus muscle (caudal pharyngeal constrictor) originates from the arch of the cricoid cartilage and inserts into the median pharyngeal raphe. The fibers of the cricopharyngeus muscle are directed dorsally, rostrally, and medially and blend caudally with the longitudinal muscular fibers of the esophagus (Sisson, 1975a).

The stylopharyngeus muscle (dorsal pharyngeal dilator) originates from the axial aspect of the distal third of the stylohyoid bone, passes ventromedially and inserts into the dorsal pharyngeal wall between the pterygopharyngeus and palatopharyngeus muscles (Sisson, 1975a). Contraction of the stylopharyngeus muscle dilates the pharynx during swallowing to receive the food bolus from the oropharynx (Holcombe and Ducharme, 2004b; Sisson, 1975a). Similarly, contraction of the stylopharyngeus muscle pulls the pharyngeal wall dorsally to allow normal breathing and prevent dynamic pharyngeal collapse during exercise (Tessier et al., 2004). In fact, the stylopharyngeus muscle appears to be more active at the end of expiration and early inspiration in horses during treadmill exercise, suggesting a critical role in the support and patency of the nasopharynx during strenuous exercise (Tessier et al., 2005).

The mucous membrane of the nasopharynx and larynx has sensory receptors that influence upper airway patency and breathing patterns. These receptors receive afferent innervation from the trigeminal, glossopharyngeal, and vagus nerves (Hare, 1975). There are also pressure receptors within the laryngeal mucosa innervated by the superior laryngeal branches of the vagus nerve that are stimulated during exercise by airflow to stabilize and dilate the upper airway during exercise. During upper airway obstruction, the sudden increase in inspiratory tracheal and laryngeal negative pressures triggers these receptors leading to contraction of upper airway muscles and resistance to dynamic collapse (Holcombe, 2013a).

Soft palate:

The soft palate in the horse extends from the hard palate to the base of the epiglottis, separating the oral cavity ventrally from the nasopharynx dorsally (Sisson, 1975a). The soft palate has a strong aponeurosis, a muscular layer, and a protective mucosa. The soft palate is characterized by a median ridge flanked by a sagittal fold on the sides, and numerous palatine glands that open on this surface (Sisson, 1975a). On each side of the rostral portion of the soft palate there is a short thick mucosal fold that attaches to the lateral border of the tongue, known as the palatoglossal arch of the soft palate (anterior pillar of the soft palate). The soft palate is continued by a two mucosal folds (one in each side of the ventro-lateral wall of the pharynx) that join over the esophageal opening forming the palatopharyngeal arch of the soft palate (posterior pillar of the soft palate) (Sisson, 1975a). The position of the soft palate is mainly determined by the coordinated activity of following muscles: the palatinus, levator veli palatini, tensor veli palatini, and palatopharyngeus (Holcombe et al., 1999).

The levator veli palatini muscle originates from the muscular process of the petrous part of the temporal bone and the lateral lamina of the auditory tube, and passes rostrally and laterally to the tensor veli palatini muscle. The muscle then passes along the lateral wall of the nasopharynx and inserts into the soft palate. The main action of the levator veli palatini muscle is to elevate the soft palate and close the choanae during swallowing (Sisson, 1975a). Contraction of the levator veli palatini muscle can be seen during endoscopic examination of the nasopharynx if the gag reflex is stimulated (Holcombe and Ducharme, 2004b).

The tensor veli palatini muscle originates from the muscular process of the petrous part of the temporal bone, the pterygoid bone and the lateral lamina of the auditory tube. The muscle then passes rostrally and laterally to the levator muscle across the medial surface of the origin of the pterygoideus medialis, and then the tendon reflects around the hamulus of the pterygoid bone to insert into the soft palate aponeurosis. Contraction of the tensor veli palatini muscle tenses and brings the rostral portion of the soft palate toward the root of the tongue (Sisson, 1975a). Experimental bilateral transection of the tensor veli palatini tendons in horses has been shown to induce consistent instability of the rostral portion of the soft palate during treadmill exercise, but does not cause DDSP (Holcombe et al., 1997).

The palatinus muscle extends from the caudal border of the hard palate to the caudal border of the soft palate and has a bundle from it is continued a short distance into the palatopharyngeal arch. Contraction of the palatinus muscle shortens the soft palate (Sisson, 1975a). Pathology of the palatinus muscle has been implicated in the etiology of DDSP because it has been demonstrated this muscle to have histological evidence of denervation in horses previously diagnosed with DDSP (Holcombe and Ducharme, 2004b).

The palatopharyngeus muscle originates from the palatine and pterygoid bones and soft palate aponeurosis, and then passes caudally on the lateral walls of the pharynx and inserts into the thyroid cartilage and median pharyngeal raphe. Contraction of the palatopharyngeus muscle shortens the soft palate, and draws the larynx and esophagus toward the oropharynx during swallowing (Sisson, 1975a).

The tensor veli palatini muscle is innervated by the mandibular branch of the trigeminal nerve, whereas the palatinus, levator veli palatini and palatopharyngeus muscles are innervated by the pharyngeal branch of the vagus nerve. The pharyngeal branch of the vagus nerve originates from the cranial cervical ganglion and courses along the medial wall of the guttural pouch and dorsal wall of the pharynx (Sisson, 1975a). Consequently, infections within the guttural pouches can lead to neuritis of the pharyngeal branch of the vagus and secondary neuromuscular dysfunction of the soft palate (Hardy and Leveile, 2003).

Histological evaluation of the equine soft palate has shown that glandular tissue represents approximately 40% of the total volume (Richardson et al., 2006). The rest of the soft palate contains muscular tissue, located dorsally in the mid regions of the soft palate and diminishing caudally, and connective tissue which represents approximately 20-40% of the total volume (Richardson et al., 2006). In addition, the nasopharyngeal mucosa of the soft palate consists of pseudostratified columnar ciliated epithelial cells, whereas the oral mucosa consists of non-keratinized stratified squamous epithelium (Alkabes et al., 2010; Richardson et al., 2006).

Hyoid apparatus:

The hyoid bone is situated between the rami of the mandible and is attached to the styloid process of the petrous part of the temporal bones, root of the tongue, pharynx, and larynx (Hillmann, 1975). The hyoid bone consists of different bones. The basihyoid is the short transverse portion of the hyoid bone characterized by its oval shape on cross section and facets to articulate with the ceratohyoid and thyrohyoid bones (Hillmann, 1975). The lingual process, which is compressed laterally and has a thin dorsal border and a thick ventral border, projects rostrally from the basihyoid and is embedded in the root of the tongue (Hillmann, 1975). The thyrohyoids are paired flat bones that extend caudally and dorsally from both each end of the basihyoid bone and are connected to the thyroid cartilage by a short fibrocartilage (Hillmann, 1975). The ceratohyoids are paired short bones that articulate with the basihyoid bone ventrally and with the stylohyoid dorsally (Hillmann, 1975). The stylohyoids are approximately 18 to 20 cm paired flat bones that articulate with both the styloid process of the petrous part of the temporal bones and with the ceratohyoid bones (Hillmann, 1975). Lastly, the epihyoids are paired small wedge-shaped bones interposed between the ceratohyoid and stylohyoid bones. In the adult horse, the epihyoids becomes fused to the stylohyoids (Hillmann, 1975).

<u>Larynx:</u>

The larynx in the horse is a short strong tubular structure consisting of six cartilages interconnected by either cartilaginous or synovial joints and supported by different ligaments (Hare, 1975). The cricoid, thyroid, and arytenoid cartilages consist of hyaline cartilage, whereas the epiglottic, cuneiform, and corniculate cartilages consist of elastic cartilage. The thyroid and cricoid cartilages commonly undergo mineralization with aging; this process begins in the body of the cartilage and often extends toward the rest of the cartilage (Hare, 1975). The position of the larynx is variable in the live horse as the rostral half of the larynx lies between the rami of the mandible when the horse's head is in a neutral position, whereas only a small portion of the rostral aspect of the larynx lies between the rami of the mandible when the horse's head is extended (Cehak et al., 2010).

The cricoid cartilage is connected to the first tracheal ring by the cricotracheal ligament and has a narrow arch and a wide dorsal lamina from which the cricoarytenoideus dorsalis muscle originates (Hare, 1975). On either side of midline the lamina of the cricoid cartilage has an oval convex facet to articulate with the arytenoid cartilage. This cricoarytenoid synovial joint allows rotational movement of the arytenoid cartilage along its perpendicular and transverse axis. At the junction of the lamina and the arch, the cricoid cartilage also articulates with the thyroid cartilage allowing for rotational movement of the thyroid cartilage around the horizontal axis of the joint (Hare, 1975).

The thyroid cartilage has a thickened body and two lateral laminae (or wings) that form most of the lateral walls of the larynx (Hare, 1975). The characteristic features of the laminae include their rhomboid shape and slightly convex surface divided by an oblique line where the thyrohyoideus and thyropharyngeus muscles interlace (Hare, 1975). The medial surface is concave and is related to the laryngeal ventricle and intrinsic laryngeal muscles. The dorsal border of the thyroid cartilage provides attachment to the pharyngeal fascia and palatopharyngeus muscle. The thyroid cartilage articulates with the thyrohyoid bone through the rostral cornu and cricoid cartilage by the caudal cornu. The cricothyroid synovial joint allows rotation of the thyroid cartilage around the horizontal axis of the joint. The rostral border of the thyroid cartilage is also attached to the hyoid bone by thyrohyoid membrane, whereas the caudal border overlaps the arch of the cricoid cartilage and provides attachment to the cricothyroideus muscle (Hare, 1975). The thyroid cartilage is also connected to the epiglottic cartilage by the thyroepiglottic ligament (Hare, 1975).

The arytenoid cartilages, each of which is fused to the ipsilateral corniculate cartilage, articulate with the cricoid cartilage and are supported by the cricoarytenoid ligament (Hare, 1975). The vocal ligament extends on each side rostroventrally from the vocal process of the arytenoid cartilage and attach to the caudal border of the thyroid cartilage. The medial surface is slightly curved and covered by respiratory mucosa, whereas the lateral surface is also slightly curved and is separated from the lamina of the thyroid cartilage by the cricoarytenoideus lateralis and vocalis muscles (Hare, 1975). The transverse arytenoid ligament and arytenoideus transversus muscle connect dorsally both arytenoid cartilages (Dart et al., 2005). A ridge that contains the muscular process on the caudal aspect of the arytenoid cartilage separates the dorsal and lateral surfaces (Hare, 1975).

The epiglottic cartilage, which is fused to the cuneiform cartilages on either side of the base of the cartilage, projects rostrodorsally into the lumen of the nasopharynx (Hare, 1975). The epiglottic cartilage is connected to the thyroid cartilage by the thyroepiglottic ligament and basihyoid bone by the hyoepiglottic ligament. The ventricular ligament extends dorsocaudally on either side from the basal part of the lateral border of the epiglottic cartilage and from the cuneiform cartilage to the ventral border and adjacent part of the lateral surface of the arytenoid cartilage. The apex has two surfaces, a concave lingual surface and a convex pharyngeal surface (Hare, 1975).

There is a paucity of information regarding the biomechanical characteristics of the laryngeal cartilages in most species. Intuitively, the larynx is a rigid structure due to its supporting laryngeal cartilages that are highly resistant to deformation (Brown et al., 2013). Extrapolating from biomechanical data obtained from articular cartilage it appears that the collagen fibers, elastin, proteoglycans and mineralized matrix provide most of the mechanical strength of the laryngeal cartilages (Mow et al., 1980). Recently, Brown et al. (2013) developed a technique using tissue engineering to create cell-seeded constructs of cartilaginous structures that approximated the shape and size of equine epiglottic cartilages (Brown et al., 2013). In that study, there was a positive correlation between the extracellular matrix content (mainly proteoglycans) of the bioengineered cartilaginous structures in culture and the mechanical stiffness of the bioengineered cartilages created (Brown et al., 2013).

The only biomechanical investigation carried out with laryngeal tissues obtained from horses was aimed to determine the mechanical constituents and compressive mechanical properties of the cricoid and arytenoid cartilages (Passman et al., 2011). The study found equine cricoid and arytenoid cartilages to have a denser matrix and collagen content than the hyaline cartilage studied in other species (Passman et al., 2011). Also, the study reported a correlation between the compressive mechanical properties of the cricoid cartilage and age, which may be due to either mineralization of the tissue or general decrease in permeability arising from aging of the cartilage matrix. Based on this evidence, we speculate there might be a similar effect of age on the mechanical properties of the thyroid cartilage.

Tayama et al. (2001) measured some of the biomechanically important morphometric features of the laryngeal framework in humans. The study showed a considerable gender related difference in many of the geometric measurements of the cricoid, thyroid and arytenoid cartilages in humans (Tayama et al., 2001). Accepting that the shape of laryngeal cartilages can influence their biomechanical properties, variability of the morphology might have an impact on biomechanical properties of the equine laryngeal cartilage. In fact, a recent investigation showed mark variability of the morphology of the cricoid cartilage obtained from mature Thoroughbred horses (Dahlberg et al., 2011). These results suggest that the morphology of other laryngeal cartilages in the horse may also be variable among horses. This is an important clinical feature because sutures are placed in an identical fashion from horse to horse despite the potential differences in thyroid cartilage cricoid conformation.

Recent histological evaluations of the thyroid cartilages obtained from young horses conducted in our laboratory showed that the laminae of the thyroid cartilage is composed mainly by hyaline cartilage, whereas the border of the thyroid cartilage is composed by fibrocartilage (**Figures 1 and 2**). The hyaline cartilage is poorly mineralized, consequently the peripheral fibrocartilage appears to provide most of the mechanical stability to the cartilage and stiffness where the intrinsic muscles attach to the thyroid cartilage.

<u>Laryngeal musculature:</u>

The laryngeal muscles can be divided into the extrinsic and intrinsic depending on their main effect on the larynx: the extrinsic muscles move the larynx as a whole, whereas, the intrinsic muscles move the cartilages of the larynx in relationship to each other (Hare, 1975). Furthermore, the extrinsic muscles can be subdivided into those that move the larynx rostrally (thyrohyoideus, hyoepiglotticus, stylohyoideus, mylohyoideus, geniohyoideus, genioglossus, stylopharyngeus and palatopharyngeus), and those that move the larynx caudally (sternothyrohyoideus and omohyoideus) (Hare, 1975). The extrinsic muscles contribute to the nasopharynx patency by indirectly increasing its diameter (through change in size of the oropharynx or position of the larynx) and increasing the stability of the soft palate during exercise (Ducharme, 2012).

The thyrohyoideus muscle originates from the laminae of the thyroid cartilage, just rostral to the insertion of the sternothyroideus to the cartilage, and inserts into the thyrohyoid and basihyoid bones (Sisson, 1975b). The thyrohyoideus muscle can be considered the rostral continuation of the sternothyroideus muscle. If the hyoid bone is fixed contraction of the thyrohyoideus muscle moves the larynx rostrally; however, if the hyoid bone is not fixed the thyrohyoideus, sternothyroideus and omohyoideus muscles move the hyoid bone and the root of the tongue caudally (Sisson, 1975b).

The thyrohyoideus muscle is innervated by the hypoglossal nerve, and blockade of the hypoglossal nerve at the level of the ceratohyoid bone causes intermittent DDSP in exercising horses (Cheetham et al., 2009). Therefore, pathology of the thyrohyoideus muscle and hypoglossal nerve has been recently implicated in the etiopathogenesis of DDSP (Cheetham et al., 2009; Ducharme et al., 2003). In one study, the electromyographic activity of the thyrohyoideus muscle markedly decreased immediately prior to the development of DDSP in horses during treadmill exercise (Ducharme, 2001). Subsequently, experimental resection of the thyrohyoideus muscles induced intermittent DDSP in 7 out 10 horses while exercising further supporting the role of this muscle on the etiopathogenesis of DDSP (Ducharme et al., 2003). Most recently, another investigation showed that electrical stimulation of the thyrohyoideus muscle induced dorsal movement of the larynx (Zantingh et al., 2013). This information suggests that thyrohyoideus muscle dysfunction may lead to reduced ability of the thyrohyoideus muscles to maintain the larynx in a dorsal position and potentially cause intermittent DDSP in horses during exercise (Zantingh et al., 2013).

The hyoepiglotticus muscle originates from the oral surface of the epiglottic cartilage and inserts into the basihyoid bone (Hare, 1975). Contraction of the hyoepiglotticus muscle approximates the basihyoid bone to the epiglottic cartilage in horses at rest (Ducharme, 2012; Holcombe and Ducharme, 2004b). The hyoepiglotticus muscle is innervated by the hypoglossal nerve (Hare, 1975). Electromyographic activity of the hyoepiglotticus muscle in exercising horses markedly increased during inspiration and with the speed of the gait (Holcombe et al., 2002). In addition, electrical stimulation of the hyoepiglotticus muscle altered the shape of the epiglottis and displaced the cartilage ventrally, which suggests that contraction of the hyoepiglotticus muscle can increase the dimensions of the airway and stabilize the soft palate during intense breathing efforts (Holcombe et al., 2002).

The stylohyoideus is a fusiform muscle originates from the muscular angle of the dorsal extremity of the stylohyoid bone, runs nearly parallel to the thyrohyoid bone and inserts into the rostral part of the thyrohyoid bone. Its action is to draw the base of the tongue and the larynx dorsally and caudally. The muscle is innervated by the facial nerve (Sisson, 1975b).

The mylohyoideus muscle originates from the lingual surface of the mandible and inserts into the median lingual raphe extending from the symphysis of the mandible to the hyoid bone, and basihyoid and thyrohyoid bones (Sisson, 1975b). The mylohyoideus muscle forms a sling between the alveolar parts of the mandible to support the tongue and related muscles. Contraction of the muscle elevates the floor of the mouth, tongue, and thyrohyoid bone. The mylohyoideus muscle is innervated by the mylohyoid nerve which is a branch of the mandibular nerve (Sisson, 1975b).

The geniohyoideus muscle originates in conjunction with the genioglossus muscle from the medial surface of the mandible near the symphysis and inserts into the basihyoid bone. Contraction of geniohyoideus pulls the hyoid apparatus rostrally and protrudes the tongue (Sisson, 1975b). The genioglossus muscle inserts into the basihyoid and ceratohyoid bones. Contraction of the genioglossus depresses the tongue (especially of its middle portion); however, contraction of the caudal fibers causes protrusion of the tongue, whereas contraction of the rostral fibers causes retraction of the tongue (Sisson, 1975b). Both muscles are innervated by the hypoglossal nerve (Sisson, 1975b). In horses, genioglossus muscle action may be mimicked by the use of tongue-tie, which is a common practice to prevent intermittent DDSP in racehorses (Franklin et al., 2002).

The sternothyrohyoideus muscle is located along the ventral surface of the trachea. The sternothyrohyoideus muscle originates from the cartilage of the sternal manubrium and inserts into the lamina of the thyroid cartilage just caudal to the attachment of the thyrohyoideus muscle (sternothyroideus muscle), and into the basihyoid bone and lingual process of the hyoid bone (sternohyoideus muscle) (Sisson, 1975b). The sternothyrohyoideus muscle is innervated by the first and second cervical nerves, and contraction of this muscle causes caudal movement of the larynx and hyoid apparatus combined (laryngohyoid apparatus) (Sisson, 1975b). Surprisingly, experimentally induced dysfunction of the sternothyroideus muscle in horses has been shown to increase translaryngeal and tracheal inspiratory pressure and resistance during exercise

(Holcombe et al., 1994).

In a study performed in anesthetized dogs electrical stimulation of the genioglossus, geniohyoideus, and sternothyrohyoideus muscles caused upper airway dilatation; however, only genioglossus stimulation significantly increased the intraluminal negative pressure required to collapse the upper airway (Odeh et al., 1993). In other words, the effect of genioglossus contraction reduced the upper airway resistance to airflow. Therefore, it appears that relaxation of the genioglossus may impede upper airway airflow, and adequate genioglossus muscle tone improves upper airway patency in dogs, likely by dilating and stiffening the nasopharynx (Odeh et al., 1993).

The omohyoideus muscle originates from the subscapular fascia near the shoulder joint, and inserts into the basihyoid bone and lingual process together with the sternohyoideus. In its cranial part, the muscle blends with the sternohyoideus and is innervated by the ventral branch of the first cervical nerve (Sisson, 1975b). Contraction of the muscle retracts the hyoid bone and the tongue (Castro et al., 1999).

1.1. Factors that influence nasopharynx function at rest and during exercise:

Head and neck position

In resting horses with their heads in a natural position, the airflow turns approximately 90 degrees at the level of the nasopharynx (Ducharme, 2012). However, during exercise horses extend their head and neck to straighten the nasopharynx and decrease airway resistance and turbulence (Cook, 1981). Furthermore, extension of the head and neck not only facilitates airflow to the lungs, but also tends to stretch the soft tissues of the upper airway making the nasopharynx stiffer and more resistant to collapse (Ducharme, 2012). Head and neck position also influences the position of the larynx in such a way that flexed head position results in rostral advancement of the larynx (McCluskie et al., 2008).

The effects of head and neck position on upper airway mechanics have also been investigated in Standardbred horses during treadmill exercise (Petsche et al., 1995). Surprisingly, extension of the head and neck did not affect upper airway resistance or work of breathing; however, flexion of the head and neck increased inspiratory resistance by approximately 50%, but did not change expiratory resistance (Petsche et al., 1995). Therefore, dynamic upper airway obstructions, particularly those involving the nasopharynx, are often more clinically apparent when affected horses are exercised with their head and neck in a flexed position (Strand et al., 2009). Head and neck flexion during exercise has also been considered to play a major role in Norwegian Coldblooded Trotters (Fjordbakk et al., 2008; Strand et al., 2004) and Icelandic horses diagnosed with bilateral arytenoid cartilage and vocal fold collapse (Hanche-Olsen et al., 2010). It has also been proposed that the rider can affect the morphology and stability of the upper airway by inducing inadvertent head flexion and facilitating nasopharyngeal collapse or deterioration of existing pathologies (Allen et al., 2011; Davidson, 2002; Franklin et al., 2006; Van Erck, 2011).

In a more recent study, the effect of head and neck on laryngeal function at rest before and after sedation and during exercise was investigated in Warmblood horses (Go et al., 2014). The authors found no effect of head and neck flexion on the degree of laryngeal dysfunction (Go et al., 2014). However, these results differed from the findings from another study that demonstrated a causal relationship between rider intervention (inducing head and neck flexion) and laryngeal function (Van Erck, 2011).

<u>Inflammation of the upper airway:</u>

Inflammation of the upper airway, such as pharyngitis, has been implicated in the etiopathogenesis of several upper airway diseases manifested clinically at rest or during exercise (Holcombe and Ducharme, 2004a). Stabled horses are exposed to high levels of organic dust (containing antigens, endotoxins and a variety of particulates) that causes both upper and lower airway inflammation. The effect of stabling on airway inflammation in horses was recently studied using Arabian yearlings (Holcombe et al., 2001). Horses kept in a stable had more pharyngeal lymphoid hyperplasia, guttural pouch inflammation, and higher number and percentage of inflammatory cells (predominately neutrophils) in the bronchoalveolar fluid than horses kept in pasture. Not surprisingly, endoscopic evaluation of the nasopharynx during nasal occlusion induced DDSP more times in stabled horses than in pastured horses, which supports the theory that inflammation of the airway (including the nasopharynx) may predispose to DDSP (Holcombe et al., 1998; Holcombe et al., 2001). This information is particularly important for the racehorse industry because approximately 25-30% of young racehorses in training develop inflammatory airway disease (Moore et al., 1995; Sweeney et al., 1992), which it may last up to 3-4 months (Burrell et al., 1996; Moore et al., 1995).

It has been proposed that inflammation of the nasopharynx and guttural pouches can lead to transient neuritis or dysfunction of the pharyngeal branch of the vagus nerve, therefore, interfering with palatinus and palatopharyngeus muscle tone, and causing palatal stability and DDSP (Holcombe et al., 1998). Moreover, it has also been proposed that neuritis of the pharyngeal branch of the vagus or glossopharyngeal nerve can cause dysfunction of the palatopharyngeus muscles and nasopharyngeal collapse (Holcombe et al., 2007a). Also, nasopharyngeal collapse can be due to mucosal sensory dysfunction that prevents appropriate reflex contraction of the intrinsic musculature of the nasopharynx (Ducharme, 2012).

2. Dorsal displacement of the soft palate

Dorsal displacement of the soft palate is the leading cause of upper airway obstruction in racehorses, affecting 10-40% of Thoroughbreds and Standardbreds (Lane et al., 2006a; Priest et al., 2012), but also occasionally affects other breeds (Franklin et al., 2006). In horses that develop DDSP, the caudal free margin of the soft palate billows across the rima glottidis during exhalation leading to airway obstruction (Ducharme, 2012). Dorsal displacement of the soft palate has been shown to cause marked increase in expiratory impedance, reduction in minute ventilation, and hypoxemia in exercising horses (Holcombe, et al., 1998). Low frequency fluttering of the caudal border of the palate is believed to be the source of the loud expiratory 'gurgle' or 'snore' noise reported in 70-80% of affected horses (Franklin et al., 2004). However, horses may experience DDSP and not make any audible respiratory noise (Derksen et al., 2001; Lane et al., 2006b; Parente et al., 2002), likely due to variability in the stiffness of the soft palate among affected horses (Franklin et al., 2004).

Dorsal displacement of the soft palate can be preceded by palatal instability and turbulent airflow within the nasopharynx (Kannegieter and Dore, 1995; Lane et al., 2006a; Tan et al., 2005). Although frequent swallowing during exercise may temporarily halt palatal instability (Pigott et al., 2010), the instability usually returns within a few seconds and progress to intermittent DDSP (Barakzai and Hawkes, 2010).

There are two clinical presentations of DDSP: intermittent and persistent (Holcombe, et al., 1998). Intermittent DDSP is the most frequent clinical presentation and typically develops only during high-speed exercise (Holcombe and Ducharme, 2007b; Parente et al., 2002). Persistent DDSP is a rare clinical presentation but can be secondary to pharyngeal paresis or other pathologies that affect the normal position of the caudal border of soft palate underneath the epiglottic cartilage such as epiglottic entrapment or pharyngeal or subepiglottic cysts (Ortved et al., 2010).

2. 1. Etiopathogenesis

The etiopathogenesis of DDSP is not yet fully understood, however, it is likely multifactorial (Rehder et al., 1995). Several theories have been proposed based on observational and experimental studies (Ducharme, 2012). Currently, there are two main theories that can explain the etiopathogenesis of DDSP: 1) the neuromuscular dysfunction theory, which focuses on the dysfunction of the nerves and muscles involved in soft palate function, and 2) the structural theory, which focuses on the stability and proximity of the laryngohyoid apparatus to the soft palate. However, both theories might be interconnected, as some disorders affecting this fine tune between the muscles and its coordination have been reported in horses that developed DDSP during exercise (e.g. hyperkalemic periodic paralysis and equine protozoal myeloencephalopathy) (Carr et al., 1996).

Neuromuscular dysfunction theory

Functionally, there are three experimental models that have consistently induced DDSP in horses. The first model consists of blockade of the pharyngeal branch of the vagus nerve, which causes paralysis of the palatal muscles and persistent DDSP both at rest and during exercise (Holcombe et al., 1998). However, this model also causes dysphagia, which is not typically seen in horses with intermittent DDSP during exercise (Holcombe et al., 1998). The second model consists of transecting and removing the thyrohyoideus muscles, which causes intermittent collapse of the nasopharynx and intermittent DDSP during exercise (Ducharme et al., 2003). The third model consists of blockade of the hypoglossal nerve model, which causes paralysis of the geniohyoideus, genioglossus, hyoglossus, and styloglossus muscles and secondary inconsistent intermittent DSSP (Cheetham et al., 2009). The exact mode of action of the affected muscles in the last two models is unknown, but it is hypothesized that they prevent caudal and ventral movement of the larynx during exercise, a phenomenon that is corrected (at least temporarily) by swallowing (Barakzai and Dixon, 2011; Pigott et al., 2010).

Structural theory

There are lesions that can interference with the normal position of the caudal border of soft palate underneath the epiglottic cartilage such as pharyngeal or subepiglottic cysts, or subepiglottic granulomas (Barakzai and Dixon, 2011; Barakzai and Hawkes, 2010; Ducharme, 2012). Typically, surgical removal of these lesions results in immediate correction of the DDSP (Haynes et al., 1990). Therefore, the current understanding is that these space occupying lesions or masses predispose to DDSP, either by mechanically interfering with the seal between the caudal border of the soft palate and subepiglottic tissue or by causing pain that leads to DDSP (Ducharme, 2012).

Other factors implicated in the etiopathogenesis of DDSP include retraction of the tongue and laryngohyoid apparatus which increases the distance between the soft palate and the epiglottic cartilage leading to DDSP during exercise (Cook, 1981). Also, retraction of the tongue can push the soft palate dorsally over the epiglottis and cause DDSP during racing (Franklin et al., 2002). Most recently, abnormal position of the laryngohyoid apparatus has been implicated in the etiopathogenesis of DDSP. First, it was found that horses diagnosed with intermittent DDSP had a more ventral position of the basihyoid bone and the larynx (Chalmers et al., 2009). Also, horses that have a more dorsal position of their basihyoid bone after the laryngeal tie-forward (LTF) procedure had a higher chance to race successfully (Cheetham et al., 2008). Finally, horses with permanent DDSP (presumably a more severe clinical presentation of DDSP) had a more caudal position of their larynx compared to horses with intermittent DDSP (Ortved et al., 2010).

2. 2. Diagnosis

In 70-80% of horses diagnosed with DDSP, there is a history of poor performance and an abnormal expiratory respiratory noise often described as ‘snoring’ or ‘gurgling’ noise (Derksen et al., 2001; Franklin et al., 2004). Thorough endoscopic examination of the nasopharynx at rest is done to rule out nasopharyngeal inflammation or lesions that can interference with the normal position of the caudal border of soft palate underneath the epiglottic cartilage (Barakzai and Hawkes, 2010). However, intermittent DDSP is frequently a dynamic condition, therefore, definitive diagnoses require endoscopic evaluation of the nasopharynx while the horse is exercised on a treadmill (Barakzai and Dixon, 2011; Kannegieter and Dore, 1995; Lane et al., 2006a, 2006b; Parente et al., 2002; Tan et al., 2005) or a racetrack while instrumented with a telemetric endoscopic equipment (Desmaizieres et al., 2009; Franklin et al., 2008; Pollock et al., 2009).

Experimentally, evaluation of the respiratory sounds has been shown to be a useful technique to diagnose DDSP in horses (Derksen, 2003; Derksen et al., 2001; Franklin et al., 2004; Franklin et al., 2003). Horses affected by DDSP usually produce a broad-frequency expiratory sound, characterized by rapid periodicity (rattling) throughout expiration that can be picked up by a microphone mounted to the bridle (Derksen et al., 2001). However, this characteristic sound is not consistently produced in all horses affected by DDSP (Derksen et al., 2001; Lane et al., 2006b; Parente et al., 2002).

2. 3. Conservative treatments

There are several conservative treatments available for horses with DDSP, which reflects both the unknown etiopathogenesis of the disorder and the fact that most treatments have a similar success rate of approximately 53-61% to improve or restore racing capacity (Barakzai and Hawkes, 2010). The current recommendation for horses diagnosed with DDSP is to initially try conservative treatment once upper airway abnormalities (e.g. pharyngeal or subepiglottic cysts or subepiglottic granulomas) have been ruled out (Ducharme, 2012). In fact, immature 2 year-old horses affected by DDSP may improve spontaneously as their upper airway matures, therefore some veterinarians recommend resting these horses until the following racing year (Barakzai and Hawkes, 2010).

Accepting that nasopharyngeal and guttural pouch inflammation can lead to neuritis of the pharyngeal branch of the vagus nerve and neuromuscular dysfunction of the soft palate (Hobo et al., 1995; Holcombe et al., 1998; Holcombe et al., 2001), the use of systemic and topical anti-inflammatory medication can be an effective therapy (Barakzai and Hawkes, 2010; Holcombe and Ducharme, 2004a). Also, mouth breathing and frequent swallowing during exercise have been reported in horses prior to development of DDSP (Cook, 1981). This finding has led to the theory that air in the mouth and oropharynx disturbs the stabilizing effect of the sub-atmospheric pressure on the ventral surface of the soft palate (Cook, 1981). Therefore, the use of dropped or crossed nosebands and/or W-bits can be used to avoid mouth breathing and ameliorate the development of DDSP in racehorses (Barakzai and Hawkes, 2010; Holcombe and Ducharme, 2004a).

The use of tongue-tie is a very popular practice to prevent DDSP in racehorses. It is believed that pulling and tying the tongue out of the mouth prevents the root of the tongue from pushing the soft palate dorsally and the laryngohyoid apparatus from moving caudally and leading to DDSP (Barakzai and Dixon, 2005; Barakzai et al., 2009a; Franklin et al., 2002). However, experimental investigations have failed to identify a morphologic or physiologic effect from a tongue-tie on the upper airway (Beard et al., 2001; Cornelisse et al., 2001a; Cornelisse et al., 2001b). Despite this evidence, clinical studies currently support the use of tongue-tie to prevent DDSP in racehorses. In one study 2/6 horses with naturally occurring intermittent DDSP the use of a tongue-tie was curative (Franklin et al., 2002). Also, two other clinical studies have shown tongue-tie to be an effective practice to decrease the development of DDSP in racehorses (Barakzai et al., 2009a; Barakzai and Dixon, 2005; Barakzai et al., 2009c; Barakzai et al., 2009b).

The throat-support device, also known as Cornell collar, has been designed to mimic the surgical effect of the LTF procedure in horses diagnosed with DDSP (Woodie et al., 2005b). The Cornel collar positions the laryngohyoid apparatus more dorsal and rostral and has been shown to prevent DDSP during exercise in experimentally induced DDSP (Woodie et al., 2005b). However, the use of the throat-support device is only allowed in some racing jurisdictions. Presently, Kentucky, Michigan, Oregon, Louisiana and other states allow the use of Cornell collars at the racetracks (Ducharme, 2012). The use of the device is not permitted in the UK (Ducharme, 2012).

2. 4. Surgical treatments

The surgical procedures advocated to prevent or treat DDSP include palatoplasty, staphylectomy, sternothyrohyoideus myectomy, sternothyroideus tenotomy, and ventriculectomy (Barakzai and Hawkes, 2010; Ducharme, 2012). These surgical procedures have a reported success rate of 35-70% to improve or restore racing performance or capacity in horses diagnosed with DDSP (Allen et al., 2011).

Among the palatoplasty procedures, laser or thermal palatoplasty consists of ‘burning’ the oral or nasal surface of the caudal edge of the soft palate by using a diode laser or cautery unit with the goal of stiffening and reducing compliance of the soft palate (Barakzai et al., 2009b). Tension palatoplasty consists of removing an elliptical section of the soft palate mucosa and submucosa and then suturing the edges primarily (Ahern, 1993b). Clinical studies of horses affected by DDSP have shown that only 50% horses improved their racing performance after palatoplasty (Ahern, 1993a; Barakzai et al., 2009b; Reardon et al., 2008). Moreover, laser palatoplasty has recently been shown to be ineffective to stiffen and reduce the compliance of the soft palate (Alkabes et al., 2010).

Staphylectomy consists of resecting on of a portion of the caudal border of the soft palate, which was originally described as a treatment of DDSP because it was believed that affected horses had an abnormally long soft palate (Quinlan et al., 1949). However, the length of the soft palate in horses affected by DDSP is similar to that of normal horses (Ducharme, 2012). Moreover, staphylectomy may disturb the seal between the oropharynx and the nasopharynx allowing the passage of water and saliva from the oropharynx into the nasopharynx after surgery (Ortved et al., 2010). Therefore, clinicians are currently moving away from staphylectomy to treat horses diagnosed with DDSP (Ducharme, 2012).

Myectomy of the sternothyrohyoideus, with or without omohyoideus, has been described as a surgical strategy to reduce caudal retraction of the laryngohyoid apparatus and intermittent DDSP during racing (Barakzai et al., 2004; Carter et al., 1993; Cook, 1981; Smith and Embertson, 2005). Myectomy of the sternothyrohyoideus consists of transecting the muscle in the proximal cervical region; however, this procedure has lost popularity because of the less appealing cosmetic outcome associated with the lack of strap muscle at the surgery site (Ducharme, 2012). An alternative is to transect the tendon attachment of the sternothyrohyoideus to the thyroid cartilage which is a less invasive approach, faster to perform, and can be performed in the field (Llewellyn, 1997).

Combined staphylectomy and sternothyrohyoideus myectomy has a modest success rate of (60%) to resolve DDSP (Barakzai et al., 2004), which is similar to the success rate reported for staphylectomy alone (60%) (Anderson et al., 1995), or sternothyrohyoideus myectomy alone (58-59%) (Anderson et al., 1995; Harrison and Raker, 1988). The addition of laser palatoplasty to the combination of staphylectomy and sternothyrohyoideus myectomy does not appear to significantly improve the surgical success over the other procedures (Smith and Embertson, 2005).

2. 4. 1. <u>The laryngeal tie-forward procedure</u>

Dysfunction of the thyrohyoideus muscles during exercise has been identified in horses diagnosed with DDSP (Ducharme et al., 2003). In fact, experimental bilateral resection of the thyrohyoideus muscles in healthy horses consistently induced intermittent DDSP, and subsequent replacement function of the thyrohyoideus muscles with prosthetic sutures restored nasopharyngeal stability in such horses during exercise (Ducharme et al., 2003). The surgical procedure consist in anchoring the larynx in a more rostral and dorsal position to the basihyoid bone relative to its original position with paired sutures placed between the thyroid cartilage and the basihyoid bone along the orientation of the thyrohyoideus muscles (Woodie et al., 2005a).

Briefly, a non-absorbable suture is passed twice through each lamina of the thyroid cartilage with or without the use of metallic suture buttons or implants placed on the medial side of the thyroid cartilage to minimize cutting of the thyroid cartilage with sutures (Rossignol et al., 2012). Then, the ends of the suture secured in the right thyroid cartilage are passed over the dorsal aspect of the basihyoid bone with the dorsal end (the end secured in the dorsal aspect of the thyroid cartilage) of the suture exiting on the right side of the lingual process and the ventral end (the end secured in the ventral aspect of the thyroid cartilage) of the suture exiting on the left side of the lingual process. Then, the ends of the left suture (the suture secured in the left aspect of the thyroid cartilage) are passed over the dorsal aspect of the basihyoid bone with the dorsal end of the suture exiting on the left side of the lingual process and the ventral end of the suture exiting on the right side of the lingual process. The right ventral end of the suture is tied to the left ventral end of the suture over the ventral aspect of the lingual process, and the right dorsal end of the suture is tied to the left dorsal end of the suture over the ventral aspect of the lingual process (Santos et al., 2014) (**Figure 3**).

Alternatively, a slight modification to the standard LTF technique can be done to increase the breaking strength and stiffness of the LTF constructs (Santos et al., 2014). This modification consists of wrapping the suture around the transverse portion of the basihyoid bone near the knot, dissipating some of the load by friction of the suture over the bone (Santos et al., 2014) **(Figure 4)**. The use of the modified LTF pattern in horses with DDSP may increase the capacity of such surgical constructs to resist failure after surgery.

The specific mechanism by which the LTF procedure prevents intermittent DDSP has not been determined; however, it has been proposed the LTF procedure may mimic the action of the thyrohyoideus muscles preventing ventral and caudal movement of the larynx (Woodie et al., 2005a). However, the LTF is reported to move the basihyoid bone dorsally and caudally and the larynx dorsally and rostrally (Cheetham et al., 2008), which may simply increase the threshold at which DDSP occurs as the palate has to travel further dorsally in order to displace over the epiglottis (Barakzai and Hawkes, 2010).

Immediately after surgery, the position of the laryngohyoid apparatus can be assessed by endoscopy where the epiglottis should be located more rostral and dorsal than before surgery

(Ducharme, 2012). Also, radiographs can be taken with the head in an extended position (McCluskie et al., 2008), and one should observe a more rostral and dorsal position of the tip of the epiglottis and larynx on the image (Cheetham et al., 2008).

Results from clinical studies have shown that the LTF procedure is currently the best surgical option for horses diagnosed with DDSP based on the high success rate of the procedure (80%) to restore nasopharyngeal stability, and the minimal patient morbidity associated with the surgical procedure (Cheetham et al., 2008; Ortved et al., 2010; Woodie et al., 2005a). Objective evaluation of the procedure's outcome and correlation with successful postoperative performance has shown the amount of postoperative rostral and dorsal movement of the larynx to have an effect on outcome (Ortved et al., 2010). Horses with a more dorsal and less rostral laryngeal position were more likely to race postoperatively than those with less elevation (Cheetham et al., 2008). Therefore, minimizing prostheses elongation is an important consideration when choosing a suture material for LTF procedures (Santos, et al., 2014), in order to maintain adequate dorsal and rostral position of the larynx after surgery and increase the chance of postoperative racing (Cheetham et al., 2008).

Complications of the LTF procedure include intraoperative bleeding (10-11%), immediate or delayed recurrence of DDSP (6%), intra-operative suture problems (2-3%), incisional infection (1%), and incisional swelling (0.7%) according to Ducharme (Ducharme, 2013). Other reported postoperative complications include bilateral vocal cord collapse (Dart et al., 2005), epiglottic entrapment, laryngeal hemiplegia, and stylohyoid bone fracture (Ducharme, 2013). The LTF procedure was initially described by drilling a tunnel in the mid portion of the basihyoid bone to allow passage and tying of the sutures (Ducharme et al., 2003; Woodie et al., 2005a). However, this practice has been abandoned to avoid the potential risk of basihyoid bone fracture either during drilling or cyclic loading imposed by the sutures (Cheetham et al., 2008). Another potential complication is the lateral deviation of the epiglottic cartilage postoperatively, which has been seen in horses with unilateral suture failure (Ducharme, 2013).

3. Suture materials utilized for the laryngeal tie-forward procedure

Although there are several nonabsorbable high-tensile strength suture materials available, only number 5 braided polyester (Ethibond, Ethicon, Somerville, NJ) and braided polyethylene (FiberWire, Arthrex, Naples, FL) sutures have been used to perform the LTF procedure in horses affected by DDSP (Ducharme et al., 2003; Woodie et al., 2005a). However, surgeons should be aware that these suture materials have different mechanical characteristics (Santos et al., 2014) and biologic reactivity (Carr et al., 2009; Esenyel et al., 2009). Polyester sutures consist of braided polyester material coated with polybutylate, whereas polyethylene sutures consist of a polyethylene core with a jacket of polyester coated with silicone to improve handling, reduce the abrasiveness and to lessen the potential for biologic reaction (Carr et al., 2009; Kümmerle, 2012). Polyethylene sutures have greater breaking strength (approximately 2.5 times) and less elongation than polyester sutures of similar size under monotonic load (Acton et al., 2004; Santos et al., 2014). In addition, polyethylene sutures have greater stiffness and more resistant to fraying than polyester sutures (Acton et al., 2004); Wust et al., 2006).

A recent investigation of the mechanical properties of LTF surrogate constructs performed with number 5 braided polyester or braided polyethylene sutures demonstrated that the constructs performed with polyethylene sutures had higher stiffness and load at failure than polyester suture constructs (Santos et al., 2014). Also, the LTF surrogate constructs performed with polyethylene sutures had less suture elongation prior to breakage (Santos et al., 2014). These results suggest that the use of polyethylene sutures to perform the LTF procedure in horses may result in surgical constructs that can withstand higher loads with less suture elongation (Santos et al., 2014). This is an important consideration when choosing the suture material for LTF procedure in vivo because an inelastic suture should result in less postoperative retraction of the larynx, which is associated with a decrease chance of racing after surgery (Cheetham et al., 2008).

There are other polyblend polyethylene suture materials available that can potentially be used to perform the LTF procedure including Herculine (Linvatec, Largo, FL), Orthocord (DePuy Mitek, Raynham, MA), and Ultrabraid (Smith & Nephew Endoscopy, Andover, MA). All these polyblend sutures are at least twice stronger than polyester sutures and 6-30 times more resistant to fraying than polyester sutures (Wust et al., 2006).

Damage of nonabsorbable suture material during handling can affect its mechanical properties. Therefore, the mechanical performance of several experimentally damaged nonabsorbable sutures including polydioxanone sutures, polyester sutures, polyester coated with polytetrafluoroethylene sutures (Tevdek, Deknatel, Mansfield, MA), ultra-high-molecular-weight polyethylene sutures (Orthocord, Ethicon, Somerville, NJ), and polyethylene sutures (FiberWire, Arthrex, Naples, FL) have been investigated (Wright et al., 2006). The study showed that polyethylene and ultra-high-molecular-weight polyethylene sutures had a higher load at failure and tensile strength than the other suture material tested (Wright, et al., 2006).

The inflammatory reaction induced by polyester, polypropylene, and polyethylene sutures, which are commonly used in orthopedic surgery, was recently evaluated in a rabbit model (Esenyel et al., 2009). The sutures were implanted in the quadriceps muscle, patellar tendon, knee capsule, and Achilles tendon, and the resultant inflammatory response was assessed at different time-points. Polyester sutures caused severe inflammatory responses; however, polyethylene sutures caused only mild inflammatory responses (Esenyel et al., 2009). In another study, suture granuloma formation in the fascia of rabbits was evaluated at 30, 60, and 120 days for several nonabsorbable suture materials (Carr et al., 2009). Ultra-high molecular weight polyethylene (MagnumWire, Opus Medical, San Juan Capistrano, CA) and polyester (Ethibond, Ethicon, Somerville, NJ) sutures caused the more severe inflammatory responses, whereas polyethylene sutures coated with silicone (FiberWire, Arthrex, Naples, FL) was found to produce a less intense inflammatory responses (Carr et al., 2009). It is believed that the different biologic reactivity of commonly used nonabsorbable sutures is attributable to their material composition, braid characteristics, and materials used to coat the sutures (Carr et al., 2009). Therefore, polyethylene sutures are the most commonly used high-tensile strength sutures in human orthopedic surgery due to its mechanical properties and low biologic reactivity (Carr, et al., 2009; Mack et al., 2009).

4. Mechanical testing of airway surgical constructs

Mechanical testing of surgical constructs has been widely used in human and veterinary surgery because this methodology provides reliable and consistent results (Ahern and Parente, 2010; Gordon et al., 2013; Kelly et al., 2008; Mathews et al., 2004; Niehaus et al., 2013; Pfeiffer et al., 1996; Zimmer et al., 1991). Also, this methodology or approach provides valuable information regarding the mechanical properties of different surgical procedures or constructs that can be immediately applied clinically (Ahern and Parente, 2010; Kelly et al., 2008; Santos et al., 2014). Studies performed with cadaver larynges do not reflect the functional status of the larynx, but may provide us with structural information important to explain some of the complications that follow surgical procedures (Ahern and Parente, 2010).

Typically, a servohydraulic load frame and a load cell connected to a computer are used to conduct biomechanical testing of surgical materials or surgical constructs (Ahern and Parente, 2010). There are several options of servohydraulic load frames that can be used depending on the material to be tested and forces being measured. Most mechanical testing machines can determine mechanical properties of materials and components using tension, compression, flexure, fatigue, impact, torsion, and hardness tests. The mechanical testing machines provide high- and low-cycle fatigue testing, thermo-mechanical fatigue testing, and fracture mechanics (Ahern and Parente, 2010; Kelly et al., 2008; Kocabey et al., 2006; Meyer et al., 2004).

Fixation of the specimens to the machine is an important consideration. Several methods have been used to mount the specimens to be studied to the material testing machine, depending on the size and the composition of the material to be tested. Plaster of Paris, casting resins, rods of different materials, clamps, Steinmann pins, polymethyl methacrylate have all been used to allow for mechanical testing at the specific angle of distraction and to accommodate to an appropriate fit to the machine (Ahern and Parente, 2010; Baltzer et al., 2001; Grumet et al., 2009; Kelly et al., 2008). Typically the angle of distraction is determined by the surgical procedure that is being tested to closely mimic the forces that would encounter in vivo (Ahern and Parente, 2010; Kelly et al., 2008; Santos et al., 2014).

CHAPTER 3

MATERIALS AND METHODS

Load transducer construction and calibration:

In order to measure the load on the LTF sutures in cadaver horses, two load transducers were constructed from 0.95 cm diameter ABS rod stock. In each end of the rod a 1.2 mm hole was drilled to allow passage of the sutures. Two miniature 0/90^o 350Ω transducer class strain gauge rosettes (S063Q-350; Vishay Micro-Measurements; Wendell, NC) were bonded to the central arm using cyanoacrylate adhesive (M-bond; Vishay Micro-Measurements; Wendell, NC) and applied 180° apart from each other, centered axially, and 90° radially from the drilled holes (**Figure 5**). Short jumper wires were attached between the solder tabs of the strain gauges to a copper solder pad at one end of the transducer to which the lead wires were also attached. The strain gauges were coated with a polymeric sealant (M-coat A; Vishay Micro-Measurements; Wendell, NC), and the transducer and wires were covered in shrink-wrap to further protect the electrical connections and supply a physical strain break (**Figure 6**).

The strain gauges were wired in to form a full bridge using a portable data acquisition system (cDAQ-9178, Compact DAQ chassis with NI 9205 I/O module, National Instruments, Austin TX) capable of recording up to 250,000 samples per second. The strain gauges were attached such that axial and transverse gauge outputs were additive, providing an increase in sensitivity of approximately 2.7 over using a single gauge. The portable data acquisition system was connected to a laptop computer running a custom written LabVIEW software (National Instruments, Austin, TX) that allows initial bridge balancing and rapid data recording.

The strain gauges and data acquisition system were calibrated using a servohydraulic load frame (MTS, Eden Prairie, MN) fitted with a 2.2 KN load cell to correlate change in voltage with change in tension. Each load transducer was attached to the load cell and actuator with number 5 polyester sutures (Ethibond, Ethicon, Somerville, NJ). In all cases, the experiments were run in load control assuring accurate input load for the calibration. Two independent tests were conducted with the load transducers, establishing a linear relationship with a Pearson's correlation coefficient of R^2=0.993 at 10 load levels over the range of interest (22-335 N). In addition, similar testing temperatures were maintained throughout the calibration process, in order to avoid temperature related errors.

<u>Measurement of loads exerted on sutures of laryngeal tie-forwards performed in cadaver horses:</u>

Five adult (median age 11 years, range 7-20 years) horses (3 Quarter Horses and 2 Standardbreds) had a LTF procedure performed immediately after euthanasia. Each horse was placed in dorsal recumbency and a traditional surgical approach used to expose the larynx and hyoid bone. The sutures used to perform the LTF constructs were instrumented with the custom made load transducers as described below:

A number 5 polyester suture (Ethibond, Ethicon, Somerville, NJ) was passed twice through the left lamina of the thyroid cartilage ventral to the insertion of the sternothyrohyoideus muscle, and another number 5 polyester suture was passed twice through the right lamina of the thyroid cartilage in a similar fashion. The free end of the left suture was passed through the closest hole of the left load transducer and then the suture was tied to the other end of the suture after removing the attached needle. The same procedure was performed in the right side with the right load transducer. The suture loops between the strain gauges and thyroid cartilage were tied so the load transducers were approximately 1-2 cm from the caudal portion of the thyroid cartilage (**Figure 7**). A third number 5 polyester suture was passed through the furthest hole of the left load transducer, and then the free end of the suture was passed in a cranial direction over the dorsal surface of the basihyoid bone to the left side of the lingual process using a wire passer, whereas the end of the suture attached to the needle was passed in cranial direction over the dorsal surface of the basihyoid bone to the right side of the lingual process. A fourth number 5 polyester suture was passed through the furthest hole of the right load transducer and the dorsal free end of the suture was passed in a cranial direction over the dorsal surface of the basihyoid bone to the right side of the lingual process, whereas the end of the suture attached to the needle was passed in cranial direction over the dorsal surface of the basihyoid bone to the left side of the lingual process (**Figure 7**).

Following instrumentation of the sutures with the load transducers as described above a sternothyrohyoideus tenectomy was performed in each side of the larynx. Then, the head was flexed approximately 90° and the tension on the load transducers was calibrated. The sutures were simultaneously tied by two investigators and data acquisition immediately started. Lastly, the horse's head was returned to the extended position, the surgery table head support was removed, and several cycles of maximal extension of the head and neck were performed and data recorded. Afterwards the sutures and load transducers were removed and the same procedure described above performed again in 3 horses.

<u>Specimen collection and laryngeal tie-forward construct preparation:</u>

Fifty fresh larynges with the musculature left in situ were harvested from adult (median: 10 years, range: 2-28 years) horses (19 Quarter Horses, 9 Standardbreds, 6 Thoroughbreds, 5 Appendix horses, 4 Warmbloods, 3 Arabians, 2 Friesians, 1 Clydesdale, 1 Morgan) euthanatized for reasons unrelated to the study. The specimens were wrapped in sponges soaked with saline solution (0.9% NaCl) and stored at -20°C until they were thawed for 24 hours at room temperature (20°C) for testing. Immediately prior to testing, the specimens were soaked in warm water (37°C) for 10 minutes.

All constructs were tested in a servohydraulic load frame (Instron Universal Testing Instrument Model 880, Instron Co., Norwood, MA) with a 4.5 KN load cell attached to the crosshead. Each larynx was mounted to the servohydraulic load frame by using a 3.2-cm diameter acrylic rod passed through the lumen of the larynx, and firmly secured to the cricoid and thyroid cartilages with 3/16-inch Steinmann pins placed perpendicular to the long axis of the larynx (**Figure 8**). Then, the acrylic rod was mounted to the load frame and number 5 polyester (Ethibond, Ethicon, Somerville, NJ) or polyethylene (FiberWire, Arthrex, Naples, FL) sutures with or without metallic implants (3.5 mm Suture Button, Arthrex, Naples, FL) were placed between the larynx and a custom-made steel model basihyoid bone fixture mounted to the load cell. The steel model basihyoid bone fixture had similar shape and dimensions of basihyoid bones of young Standardbred racehorses (**Figure 9**). The dimensions and methods of construction of the custom steel model basihyoid bone fixture were described in a recent publication (Santos et al., 2014).

The sutures were oriented to mimic the intraoperative orientation of the prosthesis placed in horses subjected to the LTF procedure. The mean angle (40°) at which the sutures were oriented relative to the long axis of the steel fixture was similar to that determined during a preliminary study (Santos et al., 2014). Each specimen was randomly allocated to one of 5 construct groups using a random number generator (www.stattrek.com/statistics/random-number-generator.aspx). The same investigator placed and tied the sutures using a square knot followed by six single throws always maintaining the same distance (70 mm) from the caudoventral border of the thyroid cartilage to the fixture; this distance was determined from LTF procedures performed in cadaver horses (Santos et al., 2014).

For LTF constructs prepared by use of the standard technique with polyester sutures (n=10) or polyethylene sutures (n=10), 2 sutures were passed twice through the laminae of the thyroid cartilage (one in each side of the cartilage) ventral to the insertion of the sternothyrohyoideus muscle, the free ends of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture, whereas the end attached to the needle of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture (**Figure 10**).

For LTF constructs prepared by use of the modified technique with polyester sutures and metallic implants (n=10) or polyethylene sutures and metallic implants (n=10), 2 sutures were passed through the laminae of the thyroid cartilage (one in each side of the cartilage) in a dorsolateral to dorsomedial direction caudal to the oblique line, then threaded through both holes of the metallic implant and passed through the laminae of the thyroid cartilage in a ventromedial to ventrolateral direction approximately 2 mm from the first passage. Traction was then applied to the suture strands to pull the metallic implant against the medial surface of the cartilage. The free ends of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture, whereas the end attached to the needle of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture (**Figure 10**).

For LTF constructs prepared by use of the modified technique with polyester sutures but without metallic implants (n=10), 2 sutures were passed twice through the laminae of the thyroid cartilage (one in each side of the cartilage) ventral to the insertion of the sternothyrohyoideus muscle. The free ends of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture on the opposite side, wrapped counter-clockwise once around the transverse portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture. The end attached to the needle of the left and right sutures were passed under the transverse portion of the fixture on the same side, wrapped counter-clockwise once around the transverse portion of the fixture, and tied together over the junction of the vertical and horizontal portions of the fixture (**Figure 10**).

<u>Mechanical testing:</u>

The sutures were pretensioned to 10 N and then subjected to a single cycle to failure (to mimic the stress applied to the constructs during recovery from general anesthesia immediately after surgery) with the load frame operating at a rate of 5 mm/s and while being recorded with high-speed digital video camera (Miro eX4, Phantom, Wayne, NJ). For each trial the following mechanical testing outcomes were determined: load (N) at 15, 20 and 30 mm of elongation, maximal load (N) and elongation (mm) at failure, and stiffness (N/mm) which was calculated as the slope of the best-fit line through the linear portion of the 'force versus the elongation' curve. Each mechanical testing outcome was compared among the different LTF construct groups. In addition, the mode of failure (suture pullout, cartilage breakage, knot slippage, or suture breakage) for each construct tested was determined by inspecting the sutures and specimens after the test and by replaying the videos obtained during each test.

Statistical analysis

The Shapiro-Wilk test was used to determine if the data had normal distributions. ANOVA was carried out using a variance components mixed model analysis to evaluate differences in maximal load and elongation at failure and stiffness. When significant differences between groups were detected, a Tukey's HSD post hoc test for pairwise multiple comparisons was performed. Analysis of variance was used to evaluate whether age had an effect on the maximal load at failure of the different LTF constructs. Dunnett test (two-sided) was used to evaluate if the mean loads obtained from the load transducers used in the cadaver horses was statistically different than the maximal load at failure of the different LTF constructs. Fisher's exact test was used to determine if there was a statistical difference on the side of failure among the LTF constructs. In addition, a factorial ANOVA was used to determine if there was a significant difference among LTF constructs at different elongation points (15, 20, 30 mm). All analyses were performed using a statistical software package (SAS, version 9.2 for Windows, SAS Institute, Cary, NC). For all statistical analyses, values of $P \leq 0.05$ were considered significant.

CHAPTER 4

RESULTS

Loads exerted on sutures of laryngeal tie-forward constructs performed in cadaver horses:

The mean ± SD maximal load exerted on the sutures to achieve adequate rostral advancement of the larynx in cadaver horses was 18 ± 7 N (range: 11-34 N). In addition, during maximal extension of the head and neck the mean ± SD maximal load recorded was 66 ± 26 N (range: 28-131 N) (**Table 1**). In some of the tests, there was moderate disparity of the loads recorded by the load transducers, which suggests that the left and right LTF sutures were not always under equal loading.

Mechanical testing of in vitro laryngeal tie-forward constructs:

The age of the specimens used to prepare the different constructs was similar between groups (**Figure 11**). The maximal load at failure was greater for the constructs prepared with polyethylene sutures by use of the standard technique than it was for the constructs prepared with metallic implants and polyethylene sutures (P=0.027) or polyester sutures (P=0.03) by use of the modified technique (**Table 2**). The maximal load at failure between constructs prepared with polyethylene sutures by use of the standard technique and constructs prepared with polyester sutures without metallic implants by use of the standard (P=0.11) or modified (P=0.89) technique was not significantly different (**Table 2**). In addition, there was no correlation between the age of the specimens and the maximal loads at failure of the surgical constructs. However, the maximal load at failure recorded in all in vitro construct types was markedly greater than the mean loads exerted on sutures of constructs performed in the cadavers horses (P<0.001).

Load at 20 mm of elongation was greater for constructs prepared with polyethylene sutures and metallic implants by use of the modified technique than it was for the constructs prepared with polyester sutures without metallic implants by use of the standard (P=0.001) or modified (P=0.048) technique (**Table 2**). Also, load at 20 mm of elongation was greater for constructs prepared with polyethylene sutures by use of the standard technique than it was for constructs prepared with polyester sutures by use of the standard (P=0.002) or modified (P=0.05) technique (**Table 2**). However, the load recorded at 15 and 30 mm of elongation was not significantly different among the different constructs (**Table 2**).

Maximal elongation at failure was lower in constructs prepared with polyethylene sutures and metallic implants by use of the modified technique than in constructs prepared with polyethylene sutures by use of the standard technique (P=0.01) (**Table 2**). Maximal elongation at failure was also lower in constructs prepared with polyethylene sutures and metallic implants by use of the modified technique than in constructs prepared with polyester sutures without metallic implants by use of either the standard (P=0.001) or modified (P=0.003) technique (**Table 2**). However, the maximal elongation at failure between constructs prepared with metallic implants with either polyethylene or polyester sutures was not significantly different (P=0.4).

Constructs prepared with polyethylene sutures and metallic implants by use of the modified technique were stiffer than the constructs prepared with polyester sutures without the metallic implants by use of the standard (P=0.008) or modified (P=0.023) technique (**Table 2**) (**Figure 12**). However, the stiffness between the constructs prepared with polyethylene sutures was not significantly different (P=0.9).

All constructs prepared with polyethylene sutures and metallic implants by use of the modified technique failed by sutures and metallic implants pulling trough the cartilage (**Figure 13**). Five constructs prepared with polyester sutures and metallic implants by use of the modified technique failed by the sutures and metallic implants pulling through the cartilage, whereas 3 constructs broke at the knot and 2 constructs pulled through the cartilage and broke the suture at the level of the metallic implant combined. Eight constructs prepared with polyester sutures by use of the standard technique failed at the knot (**Figure 14**), whereas 2 constructs pulled through the cartilage. Nine constructs prepared with polyethylene sutures by use of the standard technique broke at the cartilage (**Figure 15**) and 1 construct pulled through the cartilage **(Figure 16)**. Eight constructs prepared with polyester sutures by use of the modified technique broke at the cartilage, whereas 2 constructs broke at the suture away from the knot (**Figure 17**). Fisher's exact test showed no statistical difference between the side (right vs. left) of suture failure regardless of the suture technique and suture material tested (P=0.43).

CHAPTER 5

DISCUSSION

The results of the study did support our first hypothesis that all in vitro constructs, regardless of the surgical technique used, would have a greater load at failure than the load recorded in the sutures of the LTF constructs performed in the cadaver horses. In fact, the maximal loads recorded at failure in all in vitro surgical constructs (439-559 N) was at least three times greater than the suture load (131 N) recorded in the cadaver horses. Although in vivo conditions cannot be fully reproduced by studies utilizing cadaveric tissues, the strength recorder at the time of failure in all vitro LTF constructs suggest that the suture materials and surgical techniques tested in our study would be suitable to perform the LTF procedure in horses affected by DDSP. However, the clinical relevance of our results for this outcome should be interpreted with caution because acute clinical failure during surgery, or recovery from general anesthesia, might not be the only mechanism of failure of LTF in horses affected by DDSP. Therefore, the results of our study should be validated with cyclic testing which may be a better method or approach to mimic the in vivo forces experienced by LTF constructs.

The aim of our study was to investigate the mechanical performance of in vitro LTF tested in single loading to failure. This was intended to simulate dynamic loading of the constructs during anesthetic recovery in horses that have undergone the LTF procedure. In our clinical experience, the LTF procedure can fail immediately after the sutures have been placed at surgery or during recovery from general anesthesia. Moreover, we have found suture failure during revision surgery only when polyester sutures had been used for the LTF procedure. We suggest that in some horses subjected to the LTF procedure the sutures may not be symmetrically loaded due to multiple factors including the mechanical properties of the sutures, method of tying the sutures, method of suture passage through the cartilage, improper placement of the sutures in the cartilage, and suture pattern used. These factors may result in lack of isometric loading of the sutures postoperatively, and consequently higher loads applied to the dorsal or ventral sutures, which may increase the risks of suture breakage and construct failure. Therefore, on the basis of our results and clinical experience we currently recommend using the polyethylene sutures placed as described for the modified LTF technique (Santos et al., 2014) because this combination appears to be mechanically superior to those performed with polyester suture or metallic implants.

The constructs performed with polyethylene sutures placed with the standard LTF technique had greater maximal load at failure than constructs performed with polyethylene or polyester sutures and the metallic implants. However, there was no statistical difference when compared with the constructs performed with polyester sutures and the standard technique. This is in contrast with a previous comparison of the mechanical properties of LTF surrogates constructs prepared by use of the standard technique with similar sized polyester or polyethylene sutures which showed that constructs prepared with polyethylene sutures were stronger and stiffer and had less elongation than constructs prepared with polyester sutures (Santos et al., 2014). We speculate that the lack of significant different strength between constructs prepared with polyethylene and polyester sutures by use of the standard technique was due to the variability in tissue characteristics and viscoelastic deformation of the thyroid cartilage. These factors may have introduced additional variability in the load required to achieve maximal elongation and construct failure. This was supported by larger standard deviations observed in the present study than in our previous mechanical investigation of LTF construct surrogates (Santos et al., 2014). Another potential reason for the lack of significant difference between suture materials may be due to the relatively small number of specimens used for the study and low statistical power for detection of significant difference among data.

Minimizing postoperative suture elongation, while not compromising construct strength, should be a primary goal when choosing sutures to perform the LTF procedure in horses affected by DDSP because a more dorsal position of the larynx achieved with the procedure is associated with an increased chance of successful postoperative racing (Cheetham et al., 2008). The maximal elongation at failure was less for constructs prepared with the modified technique and metallic implants regardless of the suture material used. This finding was likely due to the length of the suture material used to perform the constructs using the metallic implants because the resultant suture loops were much shorter than the ones of the standard technique (320 mm vs 480 mm). Shortening the suture materials allows for less elongation as demonstrated by the difference in elongation between LTF constructs performed with polyester sutures with or without metallic implants. Despite the less elongation recorded in the constructs prepared with metallic implants, these constructs had the lower maximal load at failure.

The constructs prepared with polyethylene sutures and metallic implants were stiffer than the constructs prepared with polyester sutures without metallic implants. However, the stiffness of LTF constructs prepared with polyethylene sutures was not significantly different. This result was not surprising based on the mechanical properties of the polyethylene sutures (Acton et al., 2004; Santos et al., 2014; Wright et al., 2006; Wust et al., 2006). Stiffness is calculated as the slope of the line of the best fit through the linear (ie, elastic) portion of the force-versus-elongation curve, in other words, is the extent to which the material tested resists deformation in response to an applied force (Baltzer et al., 2001). Not surprisingly, polyethylene sutures have higher stiffness, therefore display less elongation when tested, which correlated well with our current and previous findings (Santos et al., 2014), and is to be expected to behave similarly in vivo.

Evaluation of the load at different elongation points (15, 20 and 30 mm) was performed to determine if there would be any differences among the LTF constructs prior to failure as the LTF constructs in vivo are not likely subjected to the elongation and loads determined at failure during monotonic loading to failure. We found a marked increase in load at 20 mm for LTF constructs prepared with polyethylene sutures with or without metallic implants. This finding was likely due to the relatively stiffness of the polyethylene sutures. However, consideration should be given to the method of suture placement in the thyroid cartilage because the use of polyethylene sutures and metallic implants approximated 63% of the maximal load at failure at 20 mm of elongation. Surgeons should be keep in mind that sudden supraphysiological loads applied to the LTF constructs in horses recovering from general anesthesia after undergoing the surgical procedure would likely cause failure of the LTF construct.

The mode of LTF failure in horses affected by DDSP is still unknown. Therefore, one of the objectives of this study was to determine and compare the mode of failure of in vitro LTF constructs prepared with the sutures and surgical techniques similar to those used for clinical applications. Failure occurred by three methods; suture breakage, cartilage breakage, and suture pull through the cartilage. Most constructs prepared by use of the standard LTF technique with polyethylene sutures passed through the caudal border of the cartilage consistently broke at the cartilage, whereas all constructs prepared by use of the modified LTF technique with polyethylene sutures and metallic implants placed in the laminae of the cartilage consistently failed by the sutures and metallic implants pulling-out from the cartilage. These results suggest that the use of polyethylene sutures with or without metallic implants exceed that strength of the thyroid cartilage, although this is not a common complication when the LTF procedure is performed clinically (Rossignol et al., 2012). In addition, the majority of the constructs prepared by use of the standard LTF technique with polyester sutures failed at the knot, whereas most constructs prepared by use of the modified LTF technique with polyester sutures without metallic implants broke at the cartilage. These results suggest that the modification to the configuration of the LTF procedure decreases the stress concentrated at the knot through frictional dissipation, and decreased shear stress at the level of the knot, which is the weakest part of a suture loop (Ilahi et al., 2008). Because of the potential concerns with surgical failure attributable to suture breakage at the knot we have been used the modified LTF technique clinically already without apparent complications.

CHAPTER 6

CONCLUSIONS

In conclusion, the data obtained from the load transducers placed in the cadaver horses suggest that LTF procedures performed with polyethylene or polyester sutures with or without metallic implants are sufficiently strong to handle the biomechanical demands on the LTF during surgery and recovery from general anesthesia. However, the use of polyethylene sutures placed through the cartilage without metallic implants appears mechanically superior to perform the LTF procedure in vivo. In addition, wrapping polyester sutures around the transverse portion of the basihyoid bone altered the failure mode and modestly increased the mechanical strength of the constructs performed with polyester sutures. Clinically, the use of polyethylene sutures and the standard LTF technique may be more biomechanically resilient than the use of polyester sutures or metallic implants. Future research is warranted in order to further elucidate the mechanical behavior of the LTF constructs during cyclic testing.

CHAPTER 7

FIGURES AND TABLES

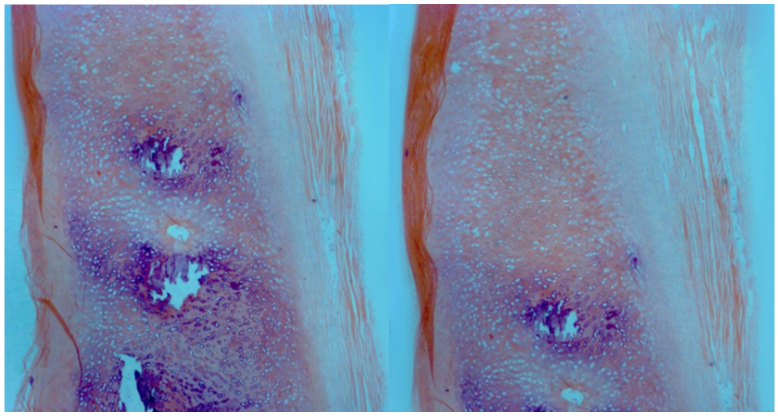

Figure 1: Histological section of the thyroid cartilage from a young horse fixed with paraformaldehyde and stained with hematoxylin and eosin. The outer surface is the right and the inner surface is to the left of the images. The sample shows a highly cellular hyaline cartilage with multifocal areas of mineralization surrounded by dense fibrocartilage. The cartilage gets thicker and more calcified as you get closer to the oblique line. Comparatively the perichondrium is thinner as you move towards the center of the cartilage and closer to the oblique line. Subjectively, the calcified hyaline cartilage accounts for most of the thicker portion of the oblique line.

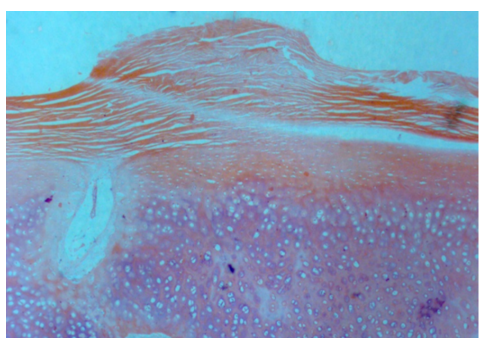

Figure 2: Histological section of the fibrocartilage at the level of the caudal border of the thyroid cartilage from the same horse above fixed with paraformaldehyde and stained with hematoxylin and eosin. Note the dense and parallel type 1 collagen bundles at the caudal border of the thyroid cartilage.

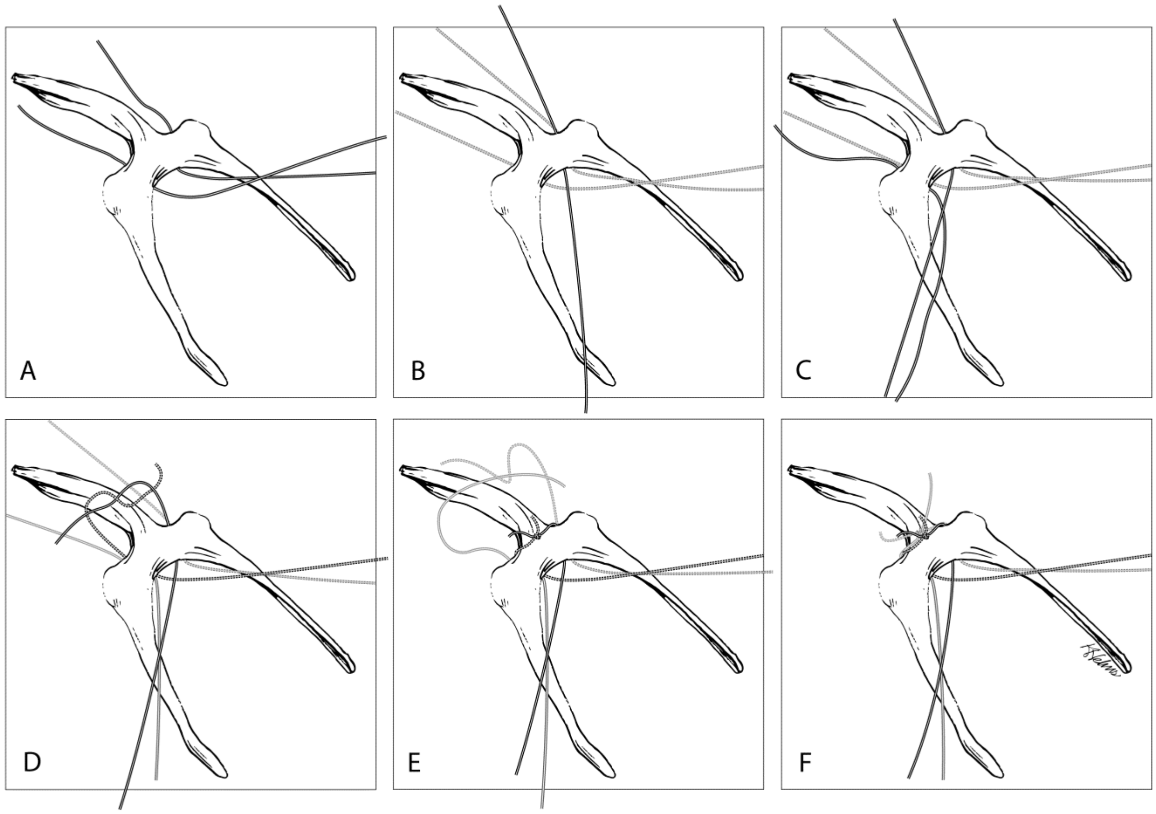

Figure 3: Illustrations showing the suture configuration around the basihyoid bone used for performance of the standard LTF procedure. After suture placement in the left and right aspects of the thyroid cartilage, the ends of the right suture (ie, the suture secured in the right aspect of the thyroid cartilage) are passed over the dorsal aspect of the basihyoid bone with the dorsal end (ie, the end secured in the dorsal aspect of the thyroid cartilage) of the suture exiting on the right side of the lingual process and the ventral end (ie, the end secured in the ventral aspect of the thyroid cartilage) of the suture exiting on the left side of the lingual process (A). Then, the ends of the left suture (ie, the suture secured in the left aspect of the thyroid cartilage) are passed over the dorsal aspect of the basihyoid bone with the dorsal end of the suture exiting on the left side of the lingual process and the ventral end of the suture exiting on the right side of the lingual process (B and C). The right ventral end of the suture is tied to the left ventral end of the suture over the ventral aspect of the lingual process (D), and the right dorsal end of the suture is tied to the left dorsal end of the suture over the ventral aspect of the lingual process (E). After the sutures are tied, both knots are positioned at the junction of the lingual process and the basihyoid bone (F)

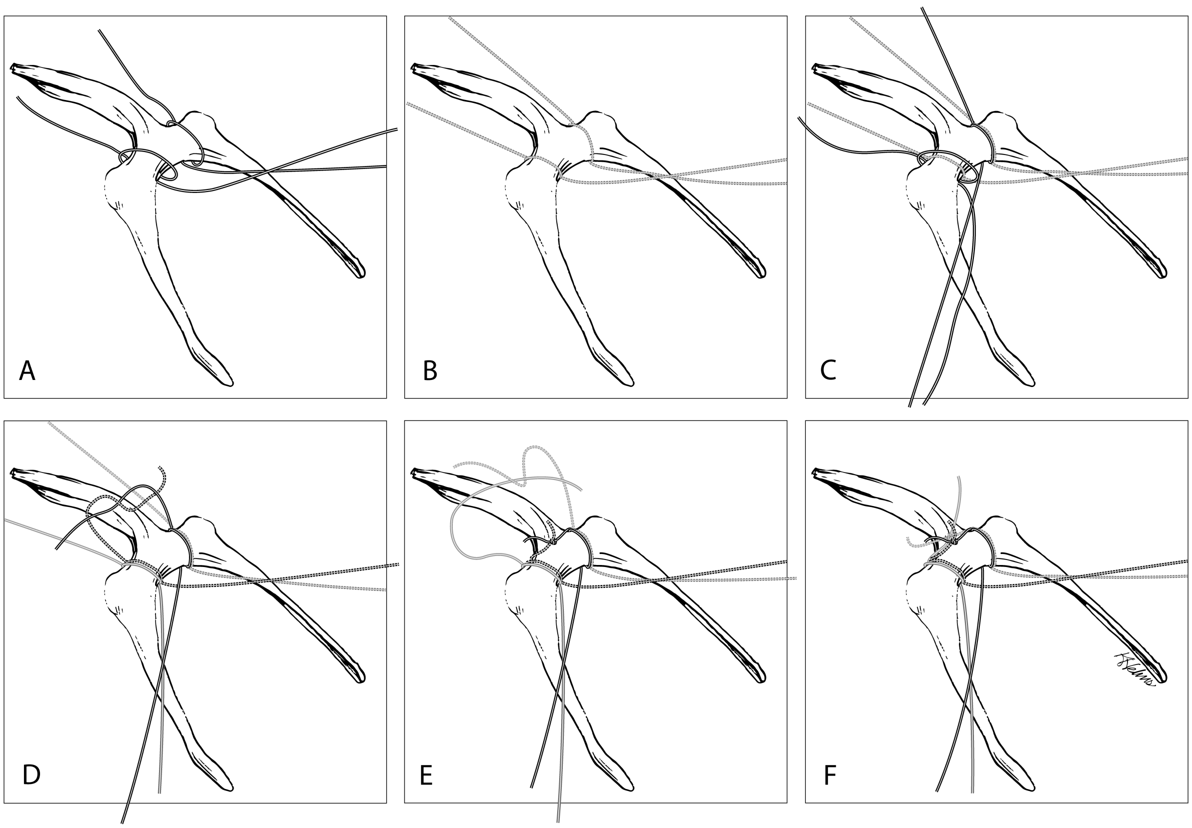

Figure 4: Illustrations showing the suture configuration around the basihyoid bone used for performance of the modified LTF procedures. The ends of the right suture are passed over the dorsal aspect of the basihyoid bone, the dorsal end is wrapped once around the basihyoid bone on the right side of the lingual process, and the ventral end is wrapped once around the basihyoid bone on the left side of the lingual process (A and B). That same procedure is performed with the left suture (C). The ventral end of the right suture is tied to the ventral end of the left suture over the ventral aspect of the lingual process (D), and the dorsal end of the right suture is tied to the dorsal end of the left suture over the ventral aspect of the lingual process (E). After the sutures are tied, both knots are positioned at the junction of the lingual process and the basihyoid bone (F).

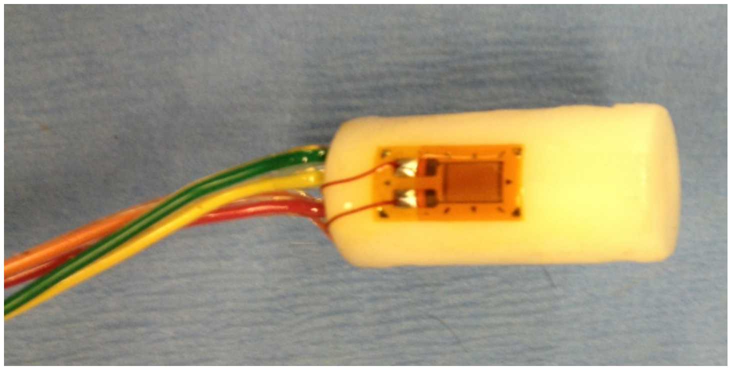

Figure 5: Image of the custom made transducer for measuring load on the LTF sutures. Note the miniature 0/90^{o} 350Ω transducer class strain gauge rosettes and short jumper wires bonded to the central arm using cyanoacrylate adhesive.

Figure 6: Image of the custom made transducer for measuring load on the LTF sutures. Note the shrink-wrap to protect the electrical connections and the holes drilled in each end of the transducer to allow passage of the sutures.

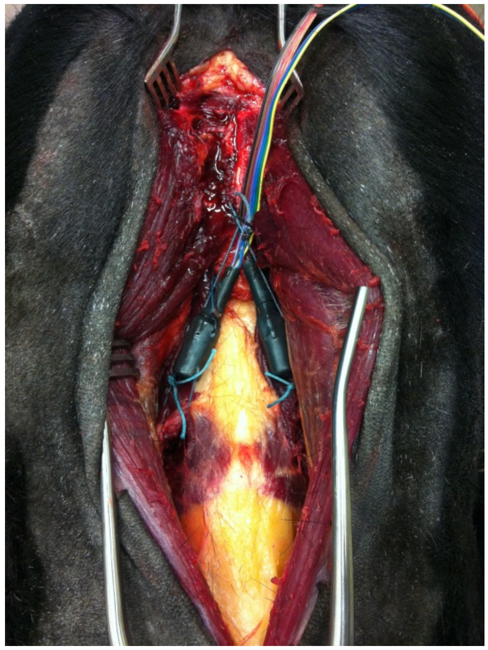

Figure 7: Image of the custom made load transducers implanted in one of the cadaver horses. Rostral is at the top of the image. Note the polyester sutures placed accordingly in order to best represent the LTF procedure.

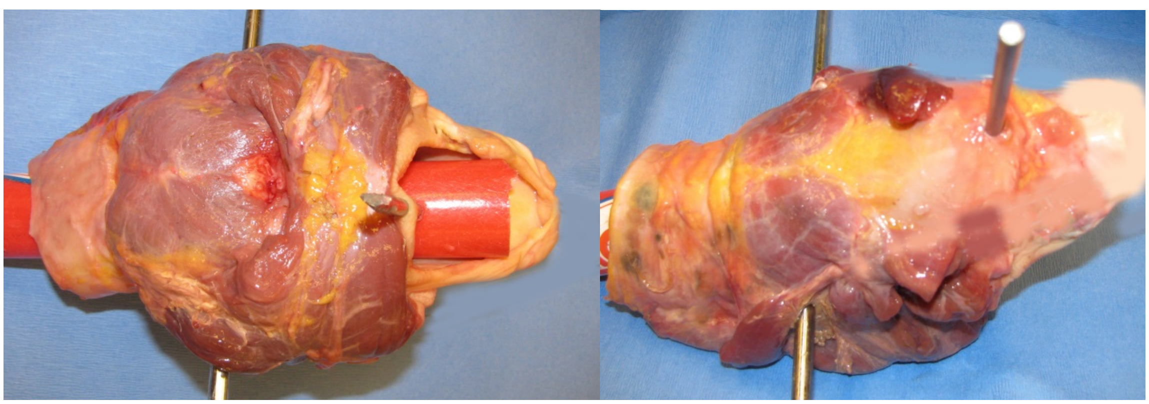

Figure 8: Images of a larynx with the musculature left in situ after being secured to the acrylic rod with Steinmann pins placed perpendicular to the long axis of the larynx. Rostral is to the right of both images.

Figure 9: Photograph of an equine basihyoid bone and a custom-made steel model basihyoid bone fixture. Notice that the short transverse bar of the model fixture is oval, whereas the base of the lingual process in the basihyoid bone is round. The width of the horizontal portion of the steel model basihyoid bone fixture was identical the distance between right and left basihyoid-ceratohyoid bone articulations in an equine cadaver examined during design of the fixture.

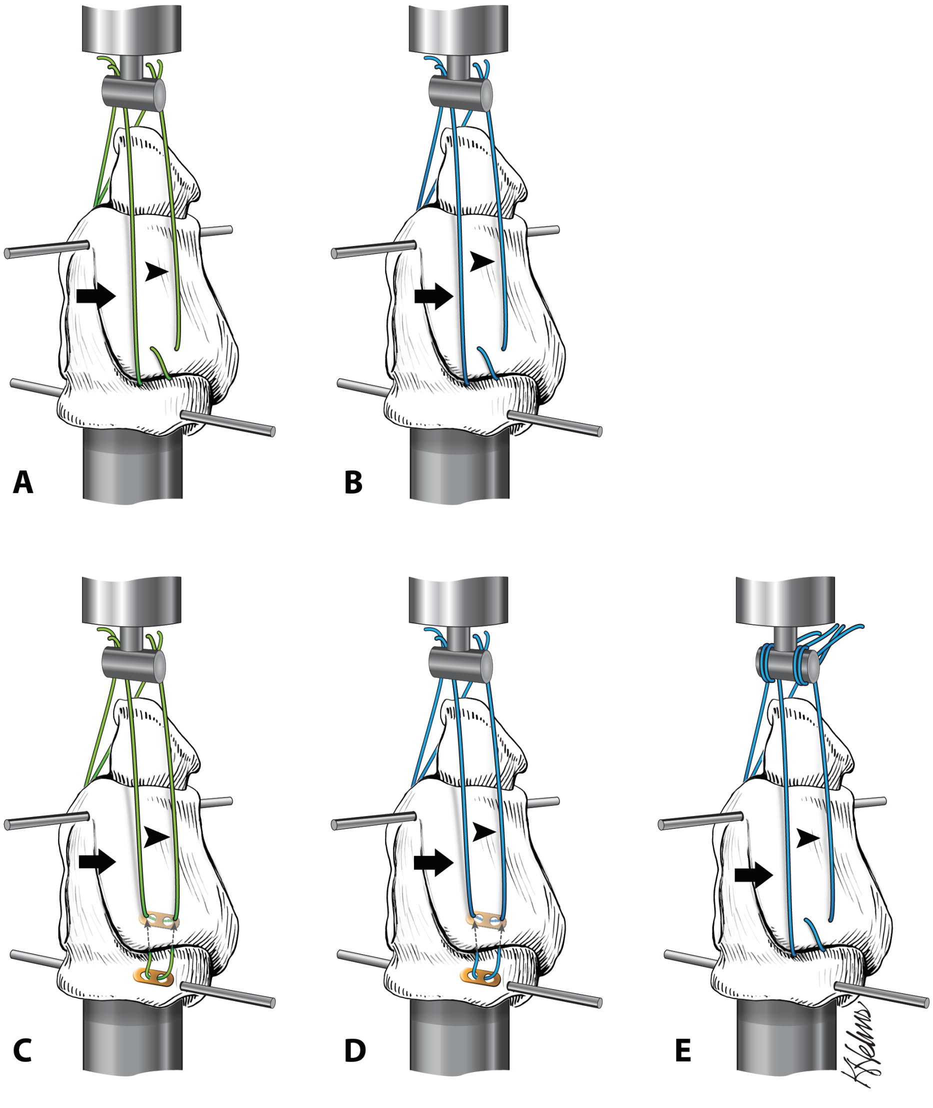

Figure 10: Illustration of the 5 suture placement patterns tested in the study. Polyester (A) or polyethylene (B) sutures were passed twice through the thyroid cartilage, the free ends (arrow) of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, whereas the end attached to the needle (arrowhead) of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the fixture. Polyester sutures and metallic implants (C) or polyethylene sutures and metallic implants (D) were passed through the thyroid cartilage in a dorsolateral to dorsomedial direction caudal to the oblique line, then threaded through both holes of the metallic implant and passed through the thyroid cartilage in a ventromedial to ventrolateral direction approximately 2 mm from the first passage. The free ends (arrow) of the left and right sutures were passed under the transverse portion of the steel model basihyoid fixture exiting on the contralateral side of the vertical portion of the fixture, whereas the end attached to the needle (arrowhead) of the left and right sutures were passed under the transverse portion of the fixture exiting on the same side of the vertical portion of the

fixture. Polyester sutures (E) were passed twice through the thyroid cartilage, the free ends (arrow) of the left and right sutures were wrapped counter-clockwise once around the transverse portion of the fixture, whereas the end attached to the needle (arrowhead) of the left and right sutures were wrapped counter-clockwise once around the transverse portion of the fixture. For all constructs the free ends of the left and right sutures were tied together over the junction of the vertical and horizontal portions of the fixture and the end attached to the needle of the left and right sutures were tied together over the junction of the vertical and horizontal portions of the fixture.

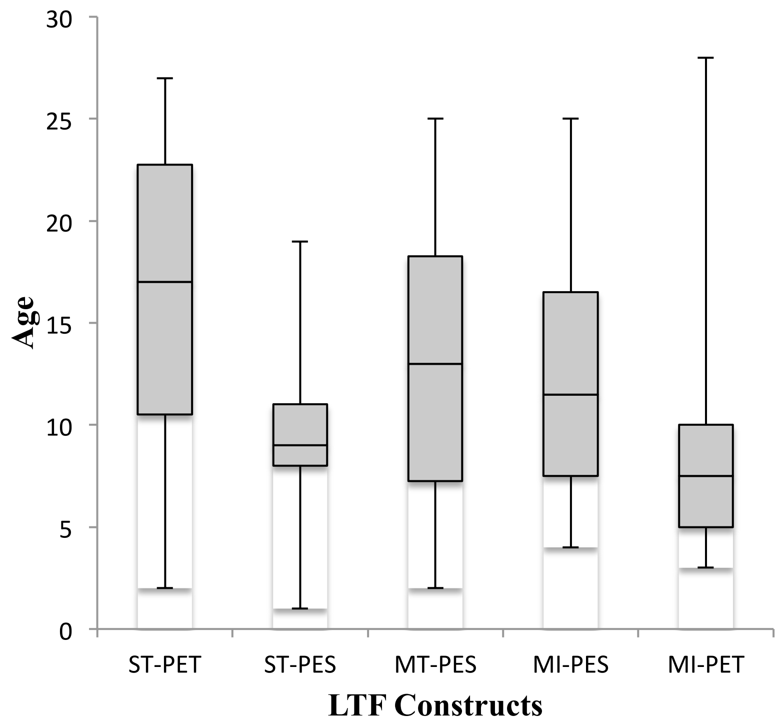

Figure 11: Box plot of mean (± SD) for age for each LTF construct group (ST-PET, standard technique polyethylene suture; ST-PES, standard technique polyester suture; MT-PES, modified technique polyester suture; MI-PES, metal implant polyester suture; MI-PET, metal implant polyethylene suture).

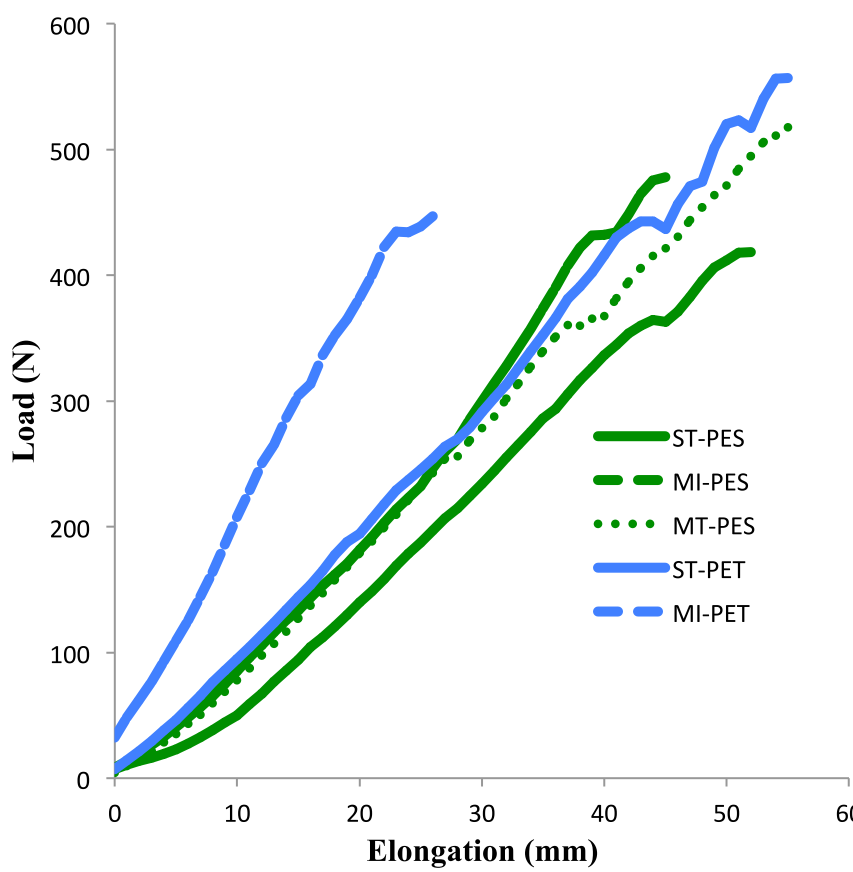

Figure 12: Typical force-elongation curve for the best representative of each of the 5 LTF constructs (ST-PET, standard technique with polyethylene sutures; ST-PES, standard technique with polyester sutures; MT-PES, modified technique with polyester sutures; MI-PES, modified technique with metal implants and polyester sutures; MI-PET, modified technique with metal implants and polyethylene sutures).

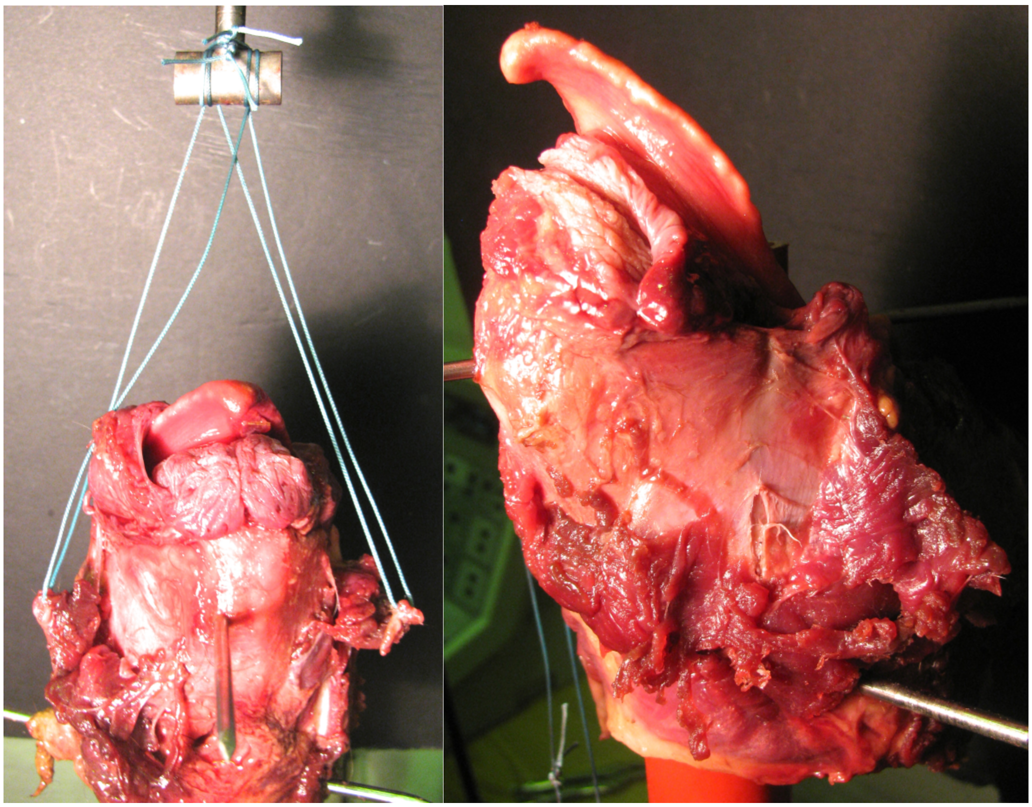

Figure 13: Images of LTF construct prepared with polyethylene sutures and metallic implants that failed by sutures and metallic implants pulling through the cartilage.

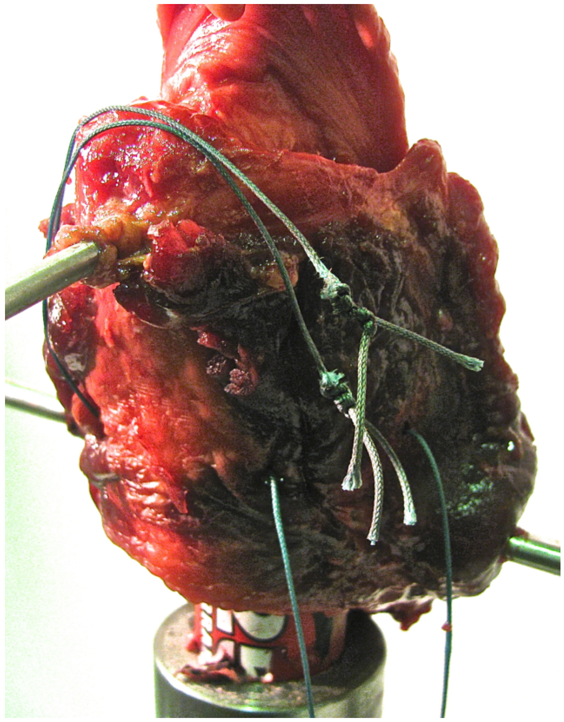

Figure 14: Image of LTF construct prepared with polyester sutures that failed by breakage of the suture at the level of the knot.

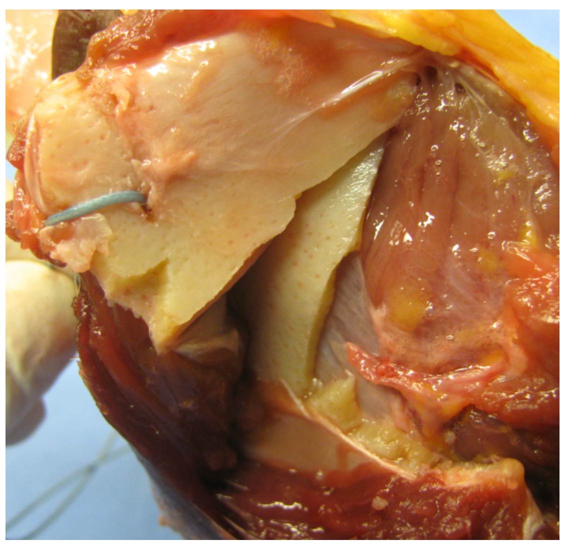

Figure 15: Image of LTF construct prepared with polyethylene sutures that failed by breakage of the cartilage.

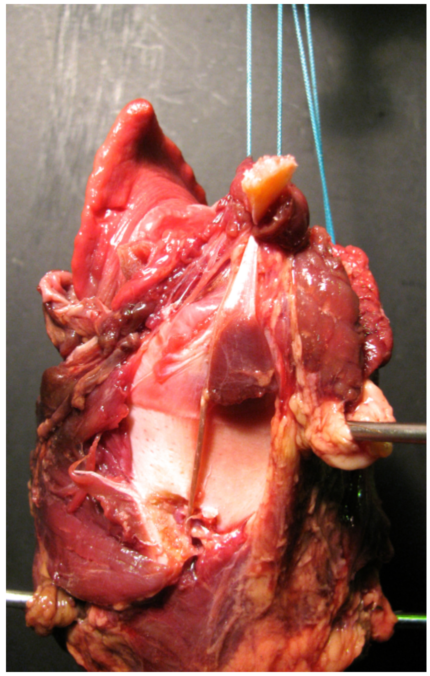

Figure 16: Image of LTF construct prepared with polyester sutures using the modified technique that failed by breakage of the cartilage.

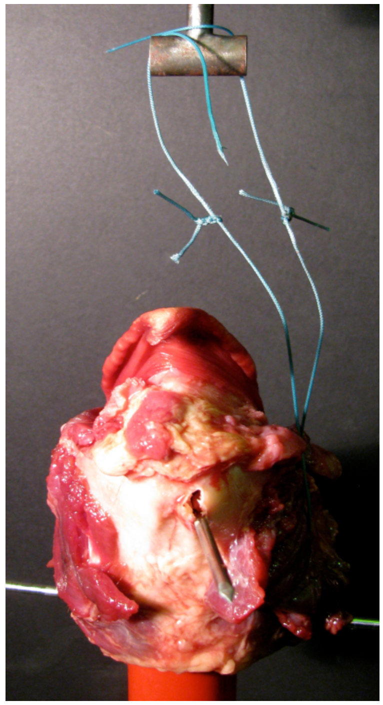

Figure 17: Image of LTF construct prepared with polyester sutures and the modified technique that failed by the breakage of suture away from the knot.

Table 1: Data collected from 5 horses instrumented with load transducers to determine the loads experienced by the LTF sutures to achieve adequate rostral advancement of the larynx and during maximal extension of the head and neck. For some horses data were recorded from 2 surgical procedures performed consequently. QH: Quarter Horse; STB: Standardbred; LT: load transducer.

Breed	Test	Load exerted on the sutures tightened after suture placement (N)	Load exerted on the sutures during maximal extension of the horse's head and neck (N)
QH	1	14.9 (LT1) 12.8 (LT2)	57.3 (LT1) 39.4 (LT2)
	2	10.8 (LT1) 12.9 (LT2)	68.4 (LT1) 63.7 (LT2)
QH	1	18.0 (LT1) 12.5 (LT2)	57.0 (LT1) 50.2 (LT2)
	2	25.0 (LT1) 18.4 (LT2)	72.0 (LT1) 70.7 (LT2)
QH	1	13.7 (LT1) 11.4 (LT2)	77.1 (LT1) 46.8 (LT2)
	2	33.6 (LT1) N/A (LT2)	130.1 (LT1) N/A (LT2)
STB	1	20.3 (LT1) 18.4 (LT2)	74.0 (LT1) 28.3 (LT2)
STB	1	30.4 (LT1) 15.1 (LT2)	107.2 (LT1) 46.1 (LT2)

Table 2: Maximal load at failure, maximal elongation at failure, load at 15, 20, and 30 mm elongation, and stiffness (Mean ± SD) for all the in vitro LTF constructs (ST-PET, standard technique with polyethylene sutures; ST-PES, standard technique with polyester sutures; MT-PES, modified technique with polyester sutures; MI-PES, modified technique with metal implants and polyester sutures; MI-PET, modified technique with metal implants and polyethylene sutures).

Surgical technique/Suture material	Maximal Load at Failure (N)	Load at 15 mm elongation (N)	Load at 20 mm elongation (N)	Load at 30 mm elongation (N)	Elongation at failure (mm)	Stiffness (N/mm)
ST-PES	469±83	157±54	192±45	299±57	54±12	0.26±0.1
MI-PES	440±82	159±15	215±20	335±36	43±9	0.29±0.2
MT-PES	523±87	157±22	206±20	316±43	52±7	0.27±0.1
ST-PET	559±114¶	206±53	277±73‡	394±103	53±10	0.33±0.1
MI-PET	439±94	214±82	279±95*	390±118	36±9#	0.37±0.1**

¶ Significantly different from MI-PET (P=0.027) and MI-PES (P=0.03) for maximal load at failure.
* Significantly different from ST-PES (P=0.001) and MT-PES (P=0.048) for load at 20 mm of elongation.
‡ Significantly different from ST-PES (P=0.002) and MT-PES (P=0.05) for load at 20 mm of elongation.
# Significantly different from ST-PES (P=0.001), MT-PES (P=0.003), and ST-PET (P=0.01) for elongation at failure.
**Significantly different from ST-PES (P=0.008) and MT-PES (P=0.023) for stiffness.

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